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DAILY GROWTH OF MAIZE

W. E. LOOMIS

(Received for publication December 11, 1932)

Increase in size in plants is the result of cell division and cell enlargement, and growth as it is commonly measured is affected by all of the internal and external factors which influence these two processes. Cell division involves the formation of protoplasm in water-saturated cells (Priestley, 1929), and the rate of cell division depends upon the supply of synthesized protoplasm building materials, upon the accelerating effect of temperature, and upon a liberal water supply *at the growing point*. Cell elongation is caused (Priestley, 1929) by the absorption of water into young, plastic-walled cells. The accumulation of sugars will increase the osmotic absorption of water, but may result in such a rapid thickening and decrease of plasticity in the walls of the elongating cells as to check the growth rate. From the nature of the reactions involved, we may assume that growth will be accelerated by: (a) quantities of synthesized protein materials with a relatively high ratio of proteins to sugars; (b) higher temperatures within the approximate range 5°C. to 35°C.; and (c) liberal moisture supplies for the active cells with their relatively low osmotic value. Growth experiments with maize have given interesting data on the effect and interrelation of temperature and moisture supply on the growth of this plant.

EXPERIMENTAL

Young maize plants 16 to 20 inches high, growing in one-gallon jars and well watered unless otherwise noted, have been used for the measurement of the rate of elongation of the youngest leaf showing from the central whorl. Home-made auxanometers were attached to the leaf tip, and growth was recorded at two-hour intervals for 24 to 48 hours. Temperature and relative humidity readings were taken at each period. The growth of three or

TABLE I. *Daily variations in the growth of maize, May, 1929*

Period	Notes	Ave. r. h. per cent	Ave. Growth mm.-hr.
8 a.m.-2 p.m. May 17	Plants in sun	40	0.79
8 a.m.-2 p.m. May 17	Plants shaded	55	1.72
2-3 p.m.	Bright sun	28	— 0.25
3-4 p.m.	Cloudy	32	2.25
5-6 p.m.	Raining	76	4.25
6 p.m.-6 a.m. May 17-18	Night	80	2.50
8 a.m.-2 p.m. May 18	Cloudy and showers	60	3.67

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four plants receiving the same treatment is averaged for each figure in the tables and graphs.

The data in table 1 indicate the wide variations in growth which may be obtained over short intervals. Between 3:00 and 4:00 p.m. on May 17 the plants were shaded by light clouds, and although the relative humidity was not appreciably affected, the growth rate rose from -0.25 mm. to $+2.25$ mm. an hour. A comparison of the growth on the mornings of May 17 and May 18 also indicates the importance of radiation in the growth rate of maize. Maize plants grew nearly five times as fast on the cloudy morning as on the sunny, while the relative humidity increased only 20 per cent. Although we commonly speak of plant growth as occurring at night and of light as inhibiting plant growth (Palladin, 1926), it appears from these data that direct sunlight is the important inhibiting factor in light. This relationship is further illustrated in figures 1 and 2, where the plants grew rapidly on the

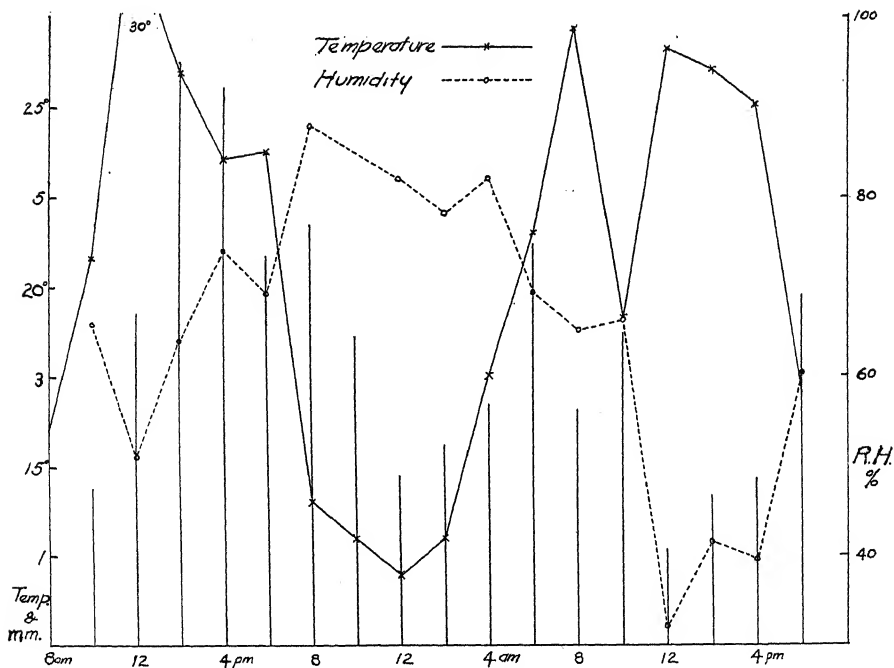


Fig. 1. Growth of maize, May, 1930. The growth rate, shown by vertical lines, follows the temperature curve at night and the humidity curve in the day.

first, a cloudy day, and reached minimum growth rates at 12:00 m. on the second, a sunny day. These graphs also indicate that the growth rate may fall or rise much more rapidly than either temperature or relative humidity with changes from shade to sun or vice versa.

These data indicate either that direct sunlight has a strongly inhibiting effect on growth through ultra-violet or other effects, or that it rapidly decreases the available water supply at the growing region by increasing the rate of evaporation from the plants. The second explanation is indicated

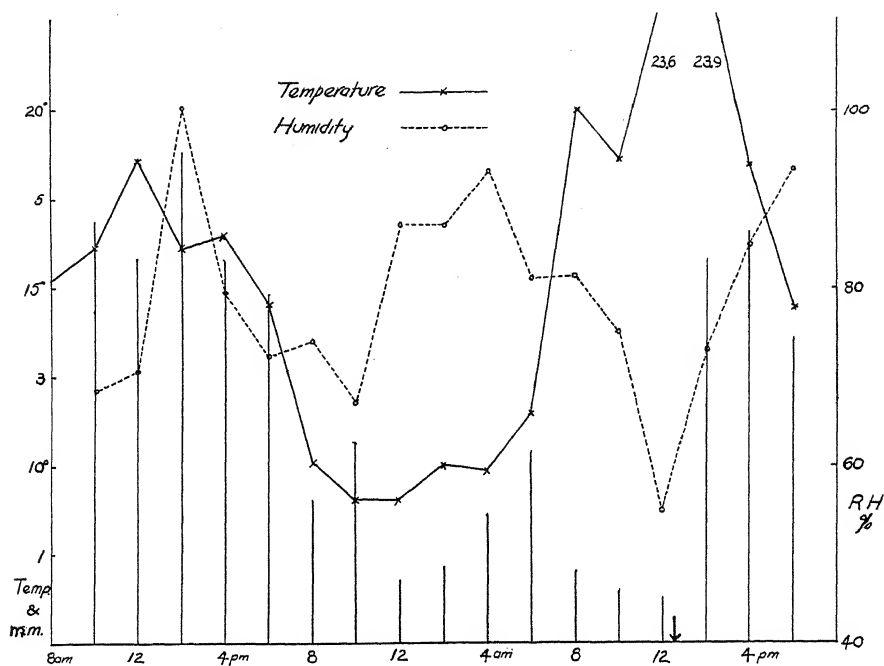


Fig. 2. Growth of maize, May, 1930. Between 8:00 a.m. and 12:00 m. on the last day of the experiment the plants were exposed to sunlight. At the point indicated by the arrow the plants were shaded with muslin.

by experiments on the rate of movement of eosin in cut stalks of maize growing in well-watered soil (table 2). The very rapid rise of eosin when these thrifty, un wilted plants were cut and transferred to the colored solution suggests that their leaf cells had built up a high water-absorbing power in the sunlight, but that this water deficit was quickly remedied if the plants were shaded for a few minutes, especially if the plants were cut and placed in water while being shaded so that the resistance of the roots to water

TABLE 2. The movement of eosin in the maize plant

Treatment	Rate of eosin movement cm.-min.
Plants growing in moist soil in bright sun	70
Plants held for 7 min. after cutting, in water in bright sun	42
Plants held for 15 min. after cutting, in water in the shade	4.1
Plants held in the shade for 15 min. before cutting	39

absorption was eliminated. With a close relationship between water supply and growth, the inhibiting effect of sunlight should be explainable on its reduction of the available water supply in the tops of the plant. Because of absorption and translocation friction this water deficit occurs in plants exposed to sunlight, even though the soil is well supplied with water, and may be expected to be rapidly effective in checking the elongation of the meristematic cells with their comparatively low osmotic value. The importance of the radiant energy of direct sunlight in comparison with the relative humidity of the air was shown by an experiment in which the water deficit of the air in grams per cubic foot was 0.329 g. in the sun and 0.333 g. in the drier, shaded room; but when plants were transferred to the shade for 20 minutes, their water deficit, as indicated by eosin movement, dropped to 24 per cent of its value in the sunlight. Incidentally, 70 cm. a minute is the most rapid rate of water movement in plants which we have seen recorded and attests to the efficiency of the maize plant.

The effect of deficient soil moisture is shown in figure 3 to be no more inhibiting to the growth of maize than is the apical water deficit induced by strong insolation. The unwatered plants wilted badly in the daytime, but their growth rate at night rose above the afternoon growth rate of the plants watered to optimum.

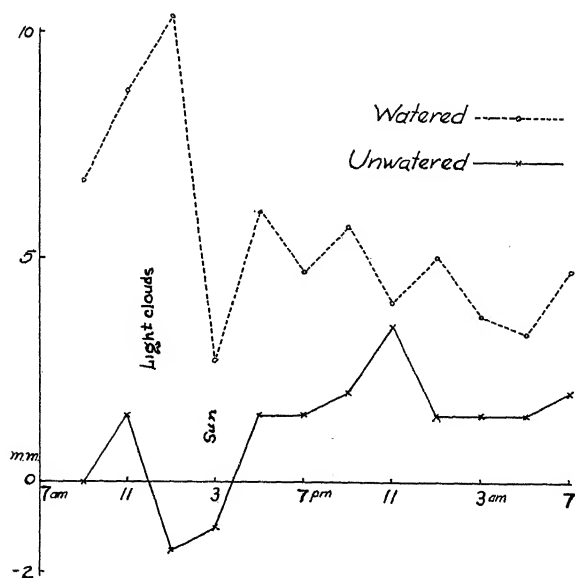


Fig. 3. Growth of maize, May, 1931. Well-watered maize made most of its growth in the warmer daylight hours of a partly cloudy day, but maize showing moderate water deficiency grew most at night.

THE RELATION OF TEMPERATURE TO THE GROWTH OF MAIZE

Although elongation and presumably the continuation of cell division depend upon the apical water supply, the cell division processes are closely correlated with temperature; and in maize, cell division approaches a minimum at about 10°C . The night growth rate in figures 1, 2, and 4 shows very clearly the effect of reduced temperature on elongation of maize. In general, the growth rates follow the temperature curve at night and the moisture-supply curve, as partially indicated by the relative humidity of the air, in the day. Maize could certainly not be expected to be a successful crop in an alpine climate with bright sunny days and cool nights.

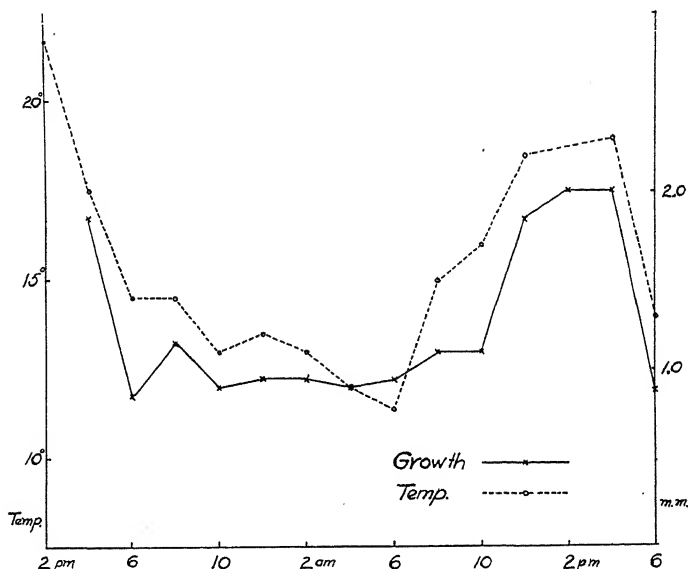


Fig. 4. Growth of maize, March, 1932. With the low temperatures and weaker sunlight of March, growth was closely correlated with temperature.

Research in progress at the present time indicates that translocation as well as cell division may be checked by low temperatures. It is generally assumed (Miller, 1924) that most of the translocation of synthesized materials from the leaves occurs at night. Our results indicate that the translocation rate in maize is highest in the afternoon, and that on cool nights translocation may stop soon after dark to be resumed at sunrise the next morning.

SUMMARY

1. The growth of maize depends upon a liberal water supply at the growing point. In order of effectiveness such a supply is reduced and growth checked by (a) direct sunlight, (b) deficient soil moisture, (c) low relative humidity.

2. The growth of maize drops rapidly as the temperature approaches 10°C. This effect is perhaps largely due to a slowing of the chemical processes of cell division, but may in part be due to a decreased rate of translocation of food materials at the low temperatures.

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THE METHOD OF COLLODION FILMS FOR STOMATA

FRANCES L. LONG AND FREDERIC E. CLEMENTS

(Received for publication December 21, 1932)

In connection with the experimental study of adaptation, it is essential to make use of comparative measurements on the widest possible scale. As an index of modification in the leaf, in terms of both growth and structure, stomata have been found to possess peculiar advantages. They have received increasing attention in consequence, and it has become important to reëxamine critically the various methods for viewing them. In earlier investigations extensive use had been made of epidermal strips supplemented by direct observation (Folsom, 1918; Loftfield, 1921), but even at its best this procedure was too time-consuming for the scrutiny of hundreds of species and forms. Furthermore, it could be applied only with difficulty or not at all to many types of leaves and but rarely permitted obtaining continuous standards from base to tip or from margin to margin. In this juncture, recourse was taken to the employment of films of collodion, with the primary purpose of increasing the rapidity of operation and the range of application. This method had already received incidental attention in connection with ecological studies at the Alpine Laboratory and offered promise of greater development.

Buscalioni and Pollacci (1902) were apparently the first to employ collodion for securing impressions of the epidermis, though the idea was derived from Berry (1891), who first utilized it for the study of striped muscle. These two investigators were attracted by the property of collodion films in clouding when moisture is present, and hoped to utilize this as a more definite and localized indicator than cobalt-chloride paper. In spite of the enthusiasm exhibited for the method, the results seem to have fallen far short of expectation, and the procedure was practically lost to view for more than two decades. However, it was employed during this period by Nathorst for fossil impressions (1908a, 1908b), and also by Naumann (1917). Further applications of the collodion method in this field were made by Lang (1926), Netolitzky (1927), Walton (1928), Barnes and Duerdon (1930), Barnes (1931), and Hoskins (1931). In addition to its use in the adaptation studies mentioned, it has been utilized by Peterson for the stomata of *Rumex acetosa* (1929), by Ashby for those of *Larrea tridentata* (1932), and by Wenzl for a series of studies dealing with technique especially (1932 in litt.)

METHODS

Solutions

Somewhat more than 150 solutions and dilutions, chiefly the latter, have been tested in the endeavor to secure the best for general use, as well as those

adapted to special purposes. In the final analysis, the number has been reduced to two compounds—namely, collodion, which is based upon cellulose nitrate, and the similar cellulose acetate. Of the various solutions employed, none has given as much satisfaction as the commercial preparations of Merck, Mallinckrodt, and Eimer & Amend. The formulae of the U. S. Pharmacopoeia likewise yield good results, but involve the small inconvenience of compounding. However, these statements apply only to simple solutions of cellulose nitrate or acetate in ether or ether-alcohol. The addition of various plasticizers, such as camphor, castor oil, and triacetin, while giving a more flexible film, is usually unfavorable to transparency and clear-cut detail. In no case have such substances proved of value in connection with the range of uses discussed later.

The rate of drying and the consistency of the film depend primarily upon the solvent and the dilution. Since they are much affected by climate and weather, especially humidity and wind, it is sometimes necessary to vary the composition as well as the dilution. The formulae of the commercial collodions are usually unavailable, and hence it is helpful for the investigator to maintain also a stock solution of his own for purposes of experimentation. This is often an advantage in connection with different types of plant material likewise. Such a solution is made from the following formula: pyroxylin, 40 g.; ether, 750 cc.; absolute alcohol, 250 cc. The greater the proportion of ether the tougher the film, while a larger amount of alcohol renders the film softer and more easily torn. However, more alcohol is an advantage in warm climates, since it slows down evaporation and causes the film to flow better.

For the most exacting work it is often desirable to take advantage of the differences between solutions of cellulose nitrate (collodion, celloidin) and cellulose acetate. Films of the latter usually give greater definition, especially in outlining apertures and revealing the finer details of the epidermis, and they are also more stable. Since the usual solvent is acetone, they dry less quickly and hence tend to wrinkle somewhat less. On the other hand, they are rather more opaque and frequently exhibit a greater tendency to cloudiness or "blushing." In consequence, commercial collodion in various dilutions is most convenient for general purposes, particularly for the number and distribution of stomata, hydathodes, hairs, etc., while acetone cellulose-acetate is to be employed for more specialized needs.

The ideal solution for stomatal films and similar purposes flows readily and hardens with due rapidity. It does not penetrate sufficiently to injure either epiderm or mesophyll, and forms a transparent, homogeneous, and fairly tough film. The latter should exhibit little tendency to become cloudy or opaque, or to wrinkle and roll at the edge. Shrinkage should be at a minimum, but this cannot be entirely eliminated without seriously affecting other more important qualities. Furthermore, it is time-saving to produce films that loosen readily at the edges, so that they can be removed quickly

and without tearing or rolling. In the majority of cases films can be made indoors to the best advantage, but it is often desirable to secure them in the field, in which case both solution and technique must be modified to meet the conditions.

Cloudiness

In the presence of small amounts of moisture, cellulose films turn white, or "blush." It was this property that appealed especially to Buscalioni and Pollacci and led to their attempt to utilize it for demonstrating the egress of water from the stomata. It is now evident that this was of slight importance and of no quantitative value, and that cloudiness in general is a distinct disadvantage, since maximum visibility is a chief desideratum. Cloudiness may be caused by water vapor escaping through the apertures, by moisture on the leaf surface, or by high humidity of the air. In leaves little or not at all cutinized, it may be produced by cuticular transpiration. If the first factors are obviated, it is not often a source of serious trouble under the microscope. Moreover, cloudiness can also be reduced by the choice of solution, collodion with a higher content of alcohol yielding the best results.

Shrinkage

From the nature of the solutions employed for making films, a small amount of shrinkage upon drying is unavoidable. The actual percentage will depend chiefly upon the kind of solution and its dilution, but is also somewhat affected by the texture of the leaf and the skill of the operator. Since the solution tends to become thicker with use, there is likewise a corresponding reduction in shrinkage, but this seems entirely negligible.

The contraction for the several solutions employed has been determined by coating an eyepiece or slide micrometer, thus securing an impression of the scale. After these films are dried under slight pressure to prevent wrinkling, the percentage of shrinkage can easily be ascertained by means of a second measurement. A number of such determinations have yielded the following averages for the several solutions, when taken from freshly opened containers:

<i>Solution</i>	<i>Shrinkage</i>
Normal collodion:	
Merck, U.S.P.	3.0%
Eimer & Amend	5.4%
Flexible collodion:	
Mallinckrodt	3.0%
Cellulose acetate (2 g. acetate, 7 g. acetone) ...	4.1%

The degree of contraction is affected not only by the texture of the leaf, but also by the nature of the surface, the presence of hairs or papillae tending to reduce the amount. Leaves with a firm texture, such as sun-forms and especially those of trees and shrubs, hold the film more rigidly when drying

and consequently minimize the shrinkage. A similar though less marked effect often obtains with films on the upper surface, owing to the greater firmness contributed by the palisade tissue. Five films from sun leaves of *Prunus demissa* gave an average shrinkage of 3.5 per cent, with a range of 3-5 per cent, while 10 films from such leaves of *Populus tremuloides* yielded a value of 3.7 per cent in every case but one.

Shade leaves with a higher sap content and a looser texture permit a greater degree of shrinkage. Such leaves tend to curl or wrinkle during the drying process, and they also become flaccid more quickly, though both tendencies can be somewhat corrected by gentle pressure on the leaf. In *Aralia nudicaulis*, the contraction was 3 per cent for the upper and 4.8 for the lower surface; *Frasera speciosa* gave 5 and 6.5, respectively, and *Heracleum lanatum* 5 and 6 per cent. These values are somewhat higher than those determined for the shrinkage of epidermal strips, which results from the release of the tension due to the turgor of the leaf. In the case of the small, firm leaf of *Chrysanthemum maximum*, the amount is low, ranging from 1 to 2.8 per cent, with an average of 1.7, but in many other types of leaf it is little if at all less than with films. Evidently, for the most accurate values, the shrinkage must be taken into account, but in general it is well within the limits of variation, even in contiguous counts.

Technique of making films

The collodion solution is applied by means of a camel's hair brush to the areas selected. In using the brush, it is important to make a single stroke whenever possible, or in the case of more, to apply these in one direction. This minimizes the disadvantage of air-bubbles and produces a smooth, thin film with the optimum impression and visibility. With many leaves and particularly on the lower surface, the film begins to loosen at the edge in a few minutes and is at once stripped off with fine forceps to prevent its rolling up. When transpiration is slight or the surface roughened in various ways, the film often adheres closely to the epiderm, and the margin must first be loosened with a sharp blade. The proper time for removing the film can only be determined by experience. If done before it is sufficiently hardened, the cells may be distorted by elongation in the direction of the pull. If removal is too long delayed, the film will curl and wrinkle to a degree that renders it unsatisfactory or worthless, especially in the case of long transects.

Leaves covered with a bloom or other waxy or resinous coating usually yield poor films and hence render it desirable or necessary to remove the wax before filming. This can easily be accomplished by washing the surface gently with ether or acetone. In the case of epiderms with hairs, the procedure differs with the form and density of these. When the trichomes form a thick layer or tomentum, a heavy coat of collodion is applied and then stripped off after a few minutes. This removes the hairs completely in most cases, though occasionally a second coating is required. As a result, the epidermis

with its stomata is fully exposed and the actual film can then be made in the usual manner. Scattered hairs commonly present no difficulty, though in some instances it is desirable to remove them by passing a razor blade obliquely over the surface.

As they are taken from the leaf, the several films from each surface are placed on a slide and covered with a second thinner slide. The latter serves the purpose of a cover-glass and at the same time permits compressing the mount by means of rubber bands at each end to prevent curling. No medium is employed for mounting, since the visibility is greatest in air. The films are allowed to harden for 24 hours and can then be examined directly. It should be borne in mind that films when first made have a somewhat fuzzy appearance, due to minute particles of the solvent, but these disappear after a few hours and the outlines become clear.

The number of slides from a particular leaf, plant, or species is often quite large, and as a result cellophane holders have proved especially convenient. They are much lighter than microscope slides, cannot be broken, require much less space for storage, and are readily transported in small paper booklets. Such holders are cut to the length of slides and twice their width. They are folded lengthwise with the films securely attached in the middle by means of bits of gummed paper. The ends are then glued together, or they may be fastened with gummed paper, which is made also to serve for a label, and are filed in paper booklets of the same size.

Though it is more convenient to make stomatal films indoors, it is sometimes desirable or necessary to do this in the field. This is especially true of thin-leaved species from shade habitats, as well as of others with rapid water loss. In communities not too sunny or exposed to the wind, this is readily done on the spot, but in prairie and desert, films are best made under shelter, a motor-car affording sufficient protection as a rule. The major precaution to be taken in field operation is to keep the collodion from drying on the brush and in the bottle. Rapid manipulation is essential to the former, and the latter is effected by utilizing a small-mouthed bottle and exposing the solution for the briefest time possible.

Counting methods and standards

While the technique of counting is simple, it makes use of several desirable accessories. The chief of these is a binocular microscope, one eyepiece of which carries the usual micrometer scale for measuring sizes and the other a counting chamber or square with an area of 1.093 sq. mm., divided into 49 smaller units. The count is recorded by means of a Veeder-Root hand-tally, with a setback knob for quickly returning to zero; the counting is always begun at the upper left-hand corner. Since the entire area yields a more accurate and representative figure, the regular practice is to enumerate all the stomata in it, but when the number is exceptionally large—e.g., 1250 to the square millimeter in *Quercus michauxi*—a single row is counted and

the result multiplied by 7. In other cases, a quarter of the area may be utilized in similar fashion, the square being marked off in four divisions by means of fine threads. When the stomata are not too crowded, 3 to 5 areas are used in order to secure a high degree of accuracy.

The wide variation in the number of stomata over the leaf of many species renders it necessary to take full account of this in devising a standard method. Apart from the well-known difference between the upper and lower surfaces, there are regularly marked divergences between various areas of the same leaf, as well as between leaves at the various levels on the same plant, sun and shade leaves of trees and shrubs especially, and between different forms or ecads of the same species. It is also of interest and of some importance to trace the shifting of stomatal numbers from the base to the tip of a leaf, and from one margin to the other. These not only bear a relation to the growth and expansion of the leaf, but are also correlated with structure and function in terms of vascular bundles and mesophyll.

In the main, the problem is much like that of determining the composition of a plant community, and hence lends itself readily to the use of minute quadrats and transects, which are conveniently termed quadrules and transules. However, in occasional species distribution is so uniform as to render numerous or consecutive counts less important.

At the outset it is essential to determine the general range of variation for each species concerned, as this will indicate the method to be employed for a comprehensive study. With leaves of small or medium size, film transules are made crosswise at two or more points and lengthwise along the median line, margin of midrib. This may be illustrated by the leaf of *Chrysanthemum maximum* (fig. 1), which has yielded the following results.

Stomatal distribution in Chrysanthemum maximum

Upper epidermis				
Margin		Midrib		
	Left	Right	Left	Right
A	31	36	29	36
B	27	33	27	28
C	30	30	30	26
D	27	29	28	27
Ave.	29	32	28	29
Lower epidermis				
A	47	51	46	49
B	50	49	46	48
C	44	39	40	40
D	34	35	39	38
Ave.	44	45	43	44

These averages were derived from 382 counts on one leaf, with extremes of 24 and 58 for the lower surface and 21 and 49 for the upper. The

number decreased rather regularly from tip to base, but the distribution from margin to midrib was less consistent (table 1).

In the case of long, narrow leaves, especially of grasses and other monocotyls, transules are run at several intervals from base to tip, while with large leaves such as those of most species of *Rumex*, a line of quadrules is located

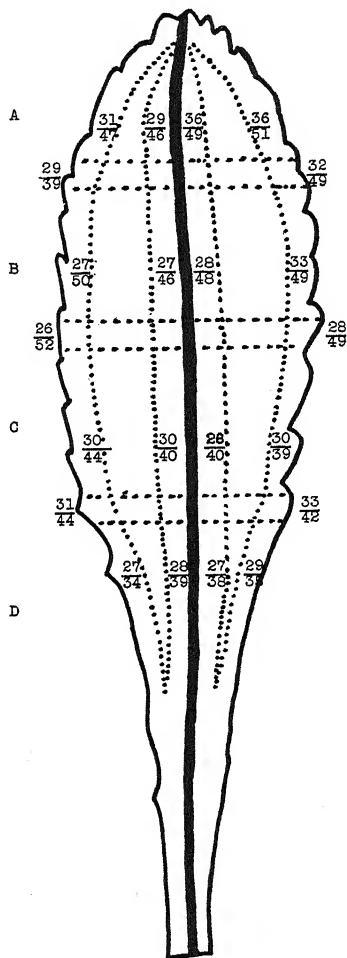


Fig. 1. Leaf of *Chrysanthemum maximum* showing transules for stomata; the respective averages indicate upper and lower epidermis by their position.

at various points. In all instances the number of films and the areas concerned are determined not only by the arrangement of the stomata and the form of the leaf, but also by the objectives in mind. Accurate results demand more films and counts than general values, a fact that applies in particular to the details of leaf adaptation to different habitats and factors.

TABLE I. *Transule counts of the stomata of Chrysanthemum maximum, with averages for each region. The interpolated numbers indicate extremes above the line and their average below, for comparison with the actual average.*

Lower epidermis				Upper epidermis							
Margin	Midrib		Margin	Margin	Midrib		Margin				
Left	Left	Right	Right	Left	Left	Right	Right				
A				A							
44	43	53	48	38	34	39	31				
46	46	58	52	37	26	39	33				
54	56	49	56	28	26	35	37				
46	45	52	43	33	27	35	39				
45	36	47	53	26	30	35	40				
48	41-54	45 36-56	45 44-58	51 41-56	27 25-38	30 25-34	42 31-42	45 31-45			
47	—	54	—	45	—	26	—	44	—		
44	47	42	46	50	51	48	48	25	—		
50	46	53	56	30	25	41	34	31	38		
49	44	46	51	34	25	33	36	31	—		
46	46	44	55	31	33	35	32	31	—		
41	46	48	54	31	33	33	—	31	—		
—	—	—	—	—	—	—	—	—	—		
47	—	46	49	51	31	29	36	36	—		
34 43 38 40 43 : 39				52 45 50 52 50 : 49				25 27 33 29 30 27 : 29		38 30 29 30 32 34 : 32	
B				B							
46	41	50	49	26	24	34	35	—	—		
47	38	50	50	25	29	37	34	—	—		
53	44	56	49	29	28	31	37	—	—		
47	40	49	48	30	24	25	33	—	—		
50	46-55	46 38-56	49 44-56	51 46-52	28 25-30	28 24-49	23 23-37	30 28-37	—		
51	—	56	—	49	—	26	—	29	—		
55	50	51	47	45	50	50	49	25	—		
52	53	44	48	25	27	25	26	28	32		
51	47	47	50	30	28	25	28	31	—		
47	40	45	46	27	25	28	34	28	—		
—	—	—	—	28	27	23	35	—	—		
50	46	48	49	27	27	28	33	—	—		
46 54 49 57 54 : 52				46 46 50 53 49 : 49				27 26 23 26 24 28 : 26		27 30 25 24 35 : 28	
C				C							
46	49	46	44	33	32	22	37	—	—		
42	49	43	51	35	36	23	33	—	—		
46	46	44	50	29	31	24	29	—	—		
45	37	44	42	37	25	26	25	—	—		
40	40-48	37 37-49	51 41-51	42 39-51	25 25-37	27 25-36	31 22-31	25 25-37	—		
48	—	44	—	41	—	44	—	30	—		
42	44	46	43	46	39	45	25	—	—		
43	43	41	41	27	31	33	30	27	31		
46	44	46	—	—	—	—	—	29	—		
—	—	—	—	—	—	—	—	—	—		
44	40	40	39	30	30	26	30	29	—		
45 45 43 42 : 44				40 46 40 42 : 42				30 34 29 30 : 31		28 37 36 31 : 33	
D				D							
44	37	38	44	31	27	31	33	—	—		
44	42	38	40	28	24	29	35	—	—		
42	42	44	39	26	32	29	33	—	—		
33	46	32	39	28	26	25	28	—	—		
37	24-44	37 33-46	44 32-44	36 26-44	30 21-31	30 24-32	24 22-31	26 26-35	—		
30	—	33	—	41	—	32	—	27	—		
26	34	—	39	38	38	30	35	29	—		
28	—	—	32	32	21	—	—	28	30		
27	—	—	28	24	—	—	—	27	—		
24	—	—	26	23	—	—	—	27	—		
—	—	—	—	—	—	—	—	28	—		
34	39	38	35	27	28	27	29	—	—		

This is well illustrated by studies on the stomata of the genus *Rumex*, in which a score of species have been taken into account, after the manner of *Rumex mexicanus*. In this, lines of quadrules were run at four points on each surface and 8-10 counts made in each area, making a total of 271 counts for the standard. This gave a maximum variation of 32 per quadrule for the upper surface and of 27 for the lower, or 19 and 29 per cent, respectively. It also revealed the fact that such comprehensive counting is often unnecessary, since the extremes regularly produced averages within one or two of those derived from the full number of counts per quadrule.

In the case of *Allium*, the leaf is usually sufficiently narrow so that transules may be made entirely around it. Variation was high as a rule in *A. cepa*, the extremes for one quadrule being 193 and 268, while from tip to base the range was 107 to 239. On the other hand, the difference between the two surfaces was slight, the grand averages being 175 and 172 for upper and lower epidermis respectively. Other succulent leaves often exhibit stomata in groups, and it is desirable to determine the spacing of the groups, as well as the varying number of stomata in these and per unit area. With leaves such as those of *Nerium*, the groups alone can be counted. For all of these, a continuous transule of greater width yields the most satisfactory results.

The quadrule method has further applications to the question of the number and fate of stomata. Not infrequently it is desired to follow the behavior of a particular region throughout a season, or from one year to another in the case of evergreen leaves or young woody shoots. For this purpose it is necessary merely to outline permanent quadrules or transules in such a manner as not to injure the epidermis, and collodion films may then be taken at any intervals wished. This method is of special value in tracing the expansion of a leaf and the fate of stomatal initials, though young leaves demand great care in placing the lines on the delicate surface.

Advantages of collodion films

Collodion films possess a number of advantages over the methods of epidermal strips or of direct vision. Chief among these are rapidity and convenience, especially under more or less trying conditions in field and garden. By contrast with stripping, they are readily applied to practically every organ and part, and they yield excellent results where strips cannot be obtained at all or only with great difficulty, as by use of the paraffin method. Such are the thin leaves of shade plants, the upper surface in many herbs, the epidermis of many shrubs and trees, especially evergreens of all sorts, of grasses and rushes, of the needles and scales of conifers, of yucca, agave, and cacti, of floating plants, and particularly of stems, fruits, and many sepals and pistils. Films alone are applicable to a wide range of hairy leaves, especially those with a dense tomentum or with numerous stellate or peltate hairs. They are also preferable because of their freedom from chlorenchyma, which is nearly always an undesirable feature of strips and peculiarly so in

micro-photography. Films are further an advantage in connection with hydathodes and lenticels, as well as in epidermal patterns and the distribution and structure of trichomes.

Collodion films are simpler to mount and file than epidermal strips and are free from the excessive clearing exhibited by epiderms mounted in balsam or similar media, especially when unstained. Their major limitation is in connection with studies of the stomatal cycle when the aperture is small or slit-like. However, in the case of stomata with large openings, as in *Chrysanthemum*, *Rumex*, or *Frasera*, for example, the use of films yields good results.

Applications of the method

As already indicated, the chief use to which the film method has been put in ecology is in connection with stomata as a ready index to adaptation, both in the field and under control. It is invaluable as a time-saver in comprehensive installations embracing a score or two of species subjected to several different factors in three or more degrees. This is equally true in the field when it is desired to obtain a composite picture of the response of dominants and subdominants in the climax or in seral stages. Its application to these on a large scale has revealed some surprising results, particularly in the case of forest layers and desert communities. It has similar value when utilized for the comparison of life-forms or for the comparative study of the species of large genera, such as *Pentstemon* and *Rumex*. It also furnishes a guide to the loss of photosynthetic activity in the case of stems, flower parts, and fruits, and reveals relict stomata in many unexpected places. Films are useful in following the behavior of the epidermis and especially of the stomata initials through the period of growth, but a painstaking special technique is required for this.

Collodion films have been frequently employed in connection with the detailed structure of fossils, and are peculiarly adapted to the study of fossil leaves and leaf-impressions. They reveal the cellular structure of some fossils remarkably well, while preliminary attempts to secure films of cross-sections of fresh stems and leaves give promise of fair success. Incidentally, they have been used to furnish good impressions of mycelium, sori, or spores in the case of certain fungi, and this application may prove to have a wide range, perhaps embracing the sori of ferns as well. As an extension of its use with foliar trichomes, the film may even come to be of value in quantitative studies of root hairs. Probably also it possesses a wide range of usefulness for animal coverings of many kinds, such as cuticle, chitin, feathers, scales, and valves, but this remains to be developed.

SUMMARY

Solutions of cellulose nitrate (collodion) and acetate are well adapted to studies of the number and distribution of stomata.

Variations in number and distribution, as well as in aperture, are most readily and accurately determined by means of film quadrules and transules.

The advantages of collodion films lie in the rapidity and convenience of operation, applicability to practically all leaf surfaces and textures, and freedom from fragments of mesophyll.

Such films possess an exceptional range of application to plant organs and parts, to various types of fossils, and to the hard parts of animals.

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AN OIL DROP THEORY OF POLLEN-GRAIN PATTERN FORMATION

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When we consider the shapes of pollen grains for the first time in our experience, we cannot fail to be struck with the great difference existing among them from the shapes which characterize the objects of our everyday experience. In fact, we find ourselves transported into a world of forms which seem remote, and have no basis of comparison among those of familiar objects, unless we except certain abstract geometrical figures. We find ourselves in a new size world, if I may be permitted the expression, in which the basic principles of form to which we are accustomed are lacking. For example, the objects with which we are familiar generally show a difference between their top and bottom sides in relation to the direction of the force of gravity, and, if they are designed by Nature or by man for motion in a horizontal direction, they generally have a front and back end and an accompanying bilateral symmetry. But among pollen grains bilateral symmetries, either with or without distinction between dorsal and ventral sides, are very rare; and, though pollen grains are great travelers, I have never seen one with its front and back ends distinguishable.

Among pollen grains radial symmetries prevail, and their forms tend ever to be spherical. Forms possessing similar attributes are found among Radiolarians and some Foraminifera, and many other objects of about the same size; and, if we wish, we may dismiss the matter by saying that pollen grains belong to a class of objects of an order of magnitude with which such symmetries are the fashion. Or we may say that they are so light that the effect of gravity is negligible, and therefore there need be no difference between their top and bottom sides; and when they travel they either stick to a moving object or drift aimlessly with the air, which supports their weight; therefore they need no front and back ends, and, relieved of the restraining influences of gravity and directional movement, they are able to achieve a much higher degree of symmetry than would otherwise be possible.

But if we analyse the matter further, we find that pollen grains and other objects of their order of magnitude really have no other choice in the matter of their form. On account of their small size their forms are restrained by two simple and well-known mathematical and physical laws. The first of these is that the surface area of an object is proportionate to the square of its linear dimensions, while its volume is proportionate to the cube of the same dimensions; which is the same thing as saying that, as the size of an object is

scaled downward, its volume decreases very much more rapidly than its surface area—in the proportion of the cube to the square—with the consequence that objects as small as pollen grains have enormous surface areas relative to their volumes.

Perhaps even more important is the second law, which governs the effect of surface tension, the elucidation of which we owe to Plateau. Surface tension we are accustomed to regard as a weak and inconsequential force, but with small plastic bodies, like developing pollen grains, it becomes a force to be reckoned with, for Plateau's law tells us that the pressure exerted by surface tension is inversely proportionate to the radius of curvature of the surface. This may be written $p = T/R$, if the curvature is only in one direction as in the case of a cylinder; but in all other objects the curvature is everywhere in two directions, so to get the full force of p they must be added together, therefore $p = T/R + T/R'$. But if we are dealing with spheres, R and R' are equal—and pollen grains are generally very nearly spherical—therefore $p = 2T/R$. We do not know T , the tension of the surface of pollen grains during their development. It probably fluctuates much and may even be different in different parts of the same grain. But whether it be great or small, it will readily be seen that a diminution in R brings about a corresponding increase in p ; and as R approaches 0 p approaches infinity. In objects as small as pollen grains the force exerted by surface tension becomes considerable because of their very sharp curvature, and this force acts upon a relatively very large area on account of their small size. In fact, it is to surface tension acting under these conditions that pollen grains owe their inherent tendency to become spherical. It is therefore reasonable to look to the action of surface tension for the explanation of some of their patterns as well as their general shape.

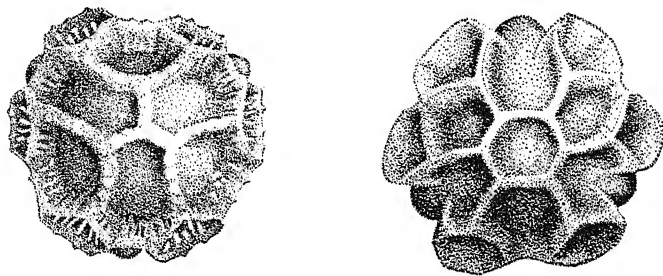


Fig. 1, 2. Fig. 1. Pollen grain of *Stokesia lacvis*, polar view. Fig. 2. Pollen grain of *Barnadesia Trianae*, polar view.

The grains of *Barnadesia*, the *Vernonieae*, and many others exhibit an elaborate system of sculpturing on their surfaces, consisting of high upstanding ridges enclosing angular spaces (fig. 1, 2). In their simpler forms the patterns assumed by these are almost geometrically regular and have a basic triradial symmetry, conforming to the three-pored form of the grains.

Such patterns can be closely imitated by blowing small soap bubbles onto a glass plate. If six bubbles are blown together there are a number of different arrangements which they may assume, one of which is shown (fig. 3), where three bubbles meet at the center of the group and three are peripheral; this pattern is almost the exact duplication of the arrangement of the lacunae of one hemisphere of the grains of *Stokesia laevis* for example (fig. 1). Or, if seven bubbles are blown together, one of the configurations which they readily assume is that shown (fig. 4) with one bubble at the center of the group surrounded by six others all in contact with it; this configuration al-

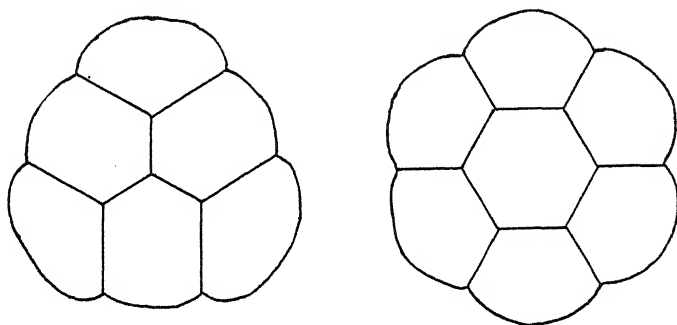


Fig. 3, 4. Fig. 3. Diagram of the arrangement of six soap bubbles blown onto a glass plate. Fig. 4. Diagram of the arrangement of seven soap bubbles blown onto a glass plate.

most exactly duplicates the lacunar pattern of the grains of *Barnadesia Trianae* (fig. 2). Or if a larger number of bubbles are blown together, they assume an arrangement which would be represented by the lacunar pattern of such grains as those of *Pacourina edulis* (Annals Bot. 42, pl. 20, fig. 18).

These pattern arrangements are not the exclusive property of soap bubbles, but are common to all liquid or plastic bodies acted upon by surface tension and free to move in one plane. The same patterns are produced by the dividing cells of some embryos when the cells are prevented from shifting into the third dimension. And Roux has demonstrated the formation of the same patterns, using drops of oil separated by a layer of viscous aqueous material.

It is a matter of common observation that in the maturing anther, pollen grains pass through the latter stages of their development bathed in a nutrient plasma which is a viscous, aqueous, colloidal emulsion bearing a fine dispersion of oil.

It has been shown by Tischler and others, and the proofs have been accepted by most investigators, that the material of the outer layer of the exine of pollen grains, which bears the sculpturing when this is present, is deposited upon the outer surface of the developing grain from the outside, and is not the product of the pollen protoplast. A most significant proof of this is that

the outer layer, with its pattern fully organized, is frequently found to develop upon the dead and empty grains of sterile hybrids.

I have observed that pollen grains which have the type of sculpturing under discussion are always found to be covered at maturity with a rather heavy coat of oil which may easily be dissolved away by ether or alcohol, while those which do not have this type of sculpturing may be without a visible coating of oil on the outside, though they may have some within.

Upon these facts the theory is here advanced that as the pollen grains develop they absorb a part of the aqueous phase of the oily emulsion by which they are surrounded; a part of the oil is consequently thrown out of suspension and condenses in droplets on the surface of the grains. These droplets, since they are separated from each other by the remaining aqueous viscous solution and are free to move, assume a least-surface configuration, which, however, is modified and controlled in spatial orientation by the spherical form of the grains and such of their contours as may be due to their three pores or furrows. With oil droplets occupying a large part of their surface, the deposition of thickening material upon the surface of the grains can take place only between the droplets; consequently the material of the outermost layer is built up in a pattern corresponding to the interstices between the droplets.

As the grain reaches maturity and dries, the oil droplets, no longer separated from each other by an aqueous viscous solution, coalesce across the separating ridges, and the grain becomes enveloped in a continuous layer of oil, which is the condition in which grains of this character are always found at maturity.

Upon this theory the pattern exhibited by a pollen grain is thus a record of the number and arrangement of the droplets of oil which condensed upon its surface during its development. When the droplets were large and few the resultant pattern is relatively simple, consisting of a few large lacunae as in the grains of *Barnadesia* and the Cichorieae. When the droplets were smaller and more numerous the resultant pattern is reticulate, as in the grains of some Vernoniaceae—for example, those of *Pacourina edulis*. Or when the droplets were very small and numerous the result is a pitted surface, as, for example, in the grains of *Rhus glabra* (Bull. Torrey Bot. Club 59, pl. 22, fig. 36). Therefore, according to this theory, the character of certain pollen-grain patterns, whether they be lacunar, reticulate, or pitted, is a function of the amount of oil present and the surface tension prevailing at its interfacial boundaries, during the formation of the grain, since these factors determine the number and size of the oil droplets which are formed on the surface.

SUMMARY

The theory is here advanced that as pollen grains develop they absorb the aqueous phase of the oily emulsion by which they are surrounded, and the oil is consequently thrown out of suspension and is condensed in droplets

upon the surface of the grains. These droplets, since they are separated from each other by a viscous aqueous solution, assume a least-surface configuration, which, however, is somewhat modified and controlled in spatial orientation by such contours of the grains as may be due to their three or more pores and furrows. With oil drops so arranged upon their surface, the deposition of the material of the outer layer upon the pollen grains can take place only between the oil droplets, consequently this material is built up in a pattern corresponding to the configuration of their interstices.

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HOST SPECIALIZATION IN THE RUST OF IRIS, *PUCCINIA IRIDIS*¹

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INTRODUCTION

The rust of iris, *Puccinia Iridis* (DC.) Wallr., is widely distributed. The Sydows (1904) list it from Europe, Asia, Japan, and North America. In North America, Arthur (1920) gives the distribution as nearly throughout the United States and Canada.

The rust is usually found in the uredinial stage. Arthur reports only uredinia east of the Rocky Mountains in the United States. The Sydows state that in many places it is found only in the uredinial stage, while in others telia appear simultaneously with the uredinia or following them in late fall or winter. Plowright (1889) notes that teliospores developed, in England, only on the species *Iris foetidissima* and *I. pseudacorus*. He states that the cultivated species, *I. flavissima*, *I. spuria*, *I. ensata*, *I. decora* (= *I. nepalensis* D. Don), *I. Kingii*, *I. pumila*, *I. filifolia*, *I. caucasica*, *I. iberica*, and *I. tolmieana* (= *I. missouriensis*), developed only uredinia.

The aecial stage has only been reported by Tranzschel (1923), who obtained aecia on *Valeriana officinalis* by inoculations from *Iris sibirica*.

The Sydows list as hosts for the species *Iris aequiloba* (= *I. pumila*), *I. caucasica*, *I. decora* (= *I. nepalensis*), *I. dichotoma*, *I. Douglasiana*, *I. ensata*, *I. filifolia*, *I. flavescens*, *I. flavissima*, *I. florentina*, *I. foetidissima*, *I. fumosa* (= *I. sindjarensis*), *I. fuscata* (? probably *I. furcata* = *I. aphylla*), *I. germanica*, *I. gracilis* (? = *I. aphylla*, or *I. prismatica* or *I. goniocarpa*), *I. graminea*, *I. Hartwegi*, *I. iberica*, *I. Kingii* (?), *I. longipetala*, *I. ochroleuca*, *I. Pallasii*, *I. pallida*, *I. pseudacorus*, *I. pseudopumila*, *I. pumila*, *I. ruthenica*, *I. spuria*, *I. tectorum*, *I. tolmieana* (= *I. missouriensis*), *I. versicolor*, *I. virginica*, and *I. xiphioides*. Although many of these species have been cultivated in the United States, Arthur reports the rust as occurring only on *Iris Douglasiana* Herb., *I. fulva* Ker., *I. longipetala* Herb., *I. missouriensis* Nutt., *I. tuberosa* L. (= *Hermodactylus tuberosus* Mill.), *I. versicolor* L., and *I. Xiphium* L.

Plowright (1889) has shown that the rust of iris in England could be separated into two groups according to host specialization. He found that

¹ This investigation was started while the writer was a member of the Department of Botany, Agricultural Experiment Station, Purdue University, and has been continued at the University of Michigan. Paper from the Department of Botany of the University of Michigan and the University Herbarium No. 398.

the rust from such species as "*Iris iberica*, *I. tolmieana*, etc." produced no effect on *I. foetidissima* and *I. pseudacorus*. He also states that Dr. M. Foster noted that the rust in his collection of *Iris* which attacked *I. fluvissima*, *I. tolmieana*, *I. iberica*, *I. spuria*, *I. ensata*, *I. decora*, *I. Kingii*, *I. pumila*, *I. filifolia*, *I. caucasica*, and species from Central Asia did not readily infect the broad-leaved Mediterranean forms such as "*I. germanica*, *I. pallida*, etc." Also, since the rust of *I. foetidissima* and *I. pseudacorus* produced telia while

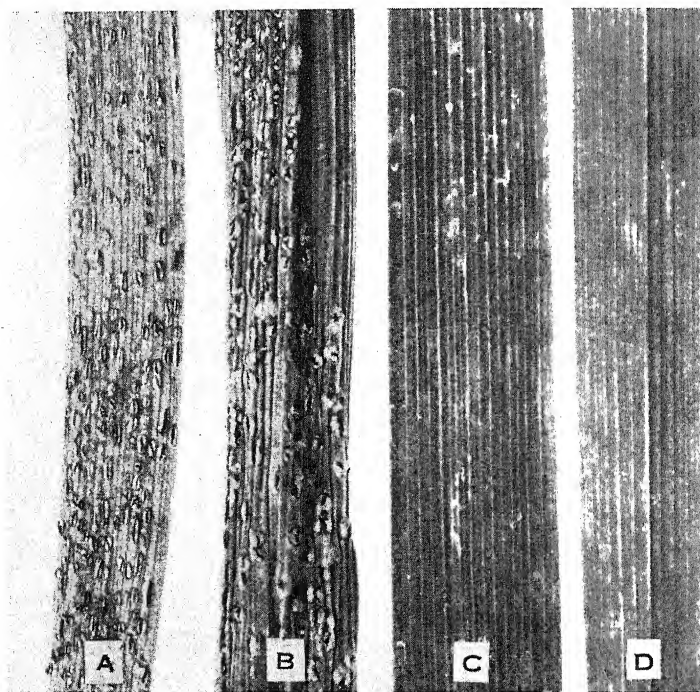


Fig. 1. Reaction to *Puccinia Iridis* sp. f. *australis*. A. *Iris missouriensis* (175), susceptible. B. *I. xiphioides* (Prince of Wales), susceptible. C. *I. Douglasiana*, highly resistant. D. *I. graminea* (X 91), highly resistant. (Reduced.)

that of the cultivated species did not, Plowright apparently concluded that he was dealing with two rusts, *Puccinia Iridis* and *Uredo Iridis*.

In 1927, Professor H. S. Jackson called the writer's attention to rust on *Iris fulva* in his garden. Since the writer was personally interested in iris and had a small collection of species in his garden, it seemed a good opportunity to study the question of host specialization. Later collections of rust on the variety Dorothea K. Williamson at Remington, Indiana, on *I. virginica* at Coldwater, Michigan, and on *I. spuria* at Ann Arbor, Michigan, made possible a comparison of the host specialization of the rust from different sources. During this investigation 33 species were studied. These may be arranged

according to the treatment in Dykes Monograph of the Genus *Iris* (1913) as follows:

Apogon Section: *Iris sibirica* L., *I. orientalis* Thunb., *I. Clarkei* Baker, *I. Bulleyana* Dykes, *I. Douglasiana* Herbert, *I. tenax* Douglas, *I. Purdyii* Eastwood, *I. foetidissima* L., *I. unguicularis* Poir. (*stylosa*), *I. spuria* L. (*I. halophila* Pallas, *I. ochroleuca* L., *I. aurea* Lindley), *I. graminea* L., *I. sintenisii* Janka, *I. Kaempferi* Siebold ex Lemaire, *I. pseudacorus* L., *I. versicolor* L. (*I. virginica* L.),² *I. hexagona* Walt., *I. foliosa* Mackenzie & Bush, *I. fulva*

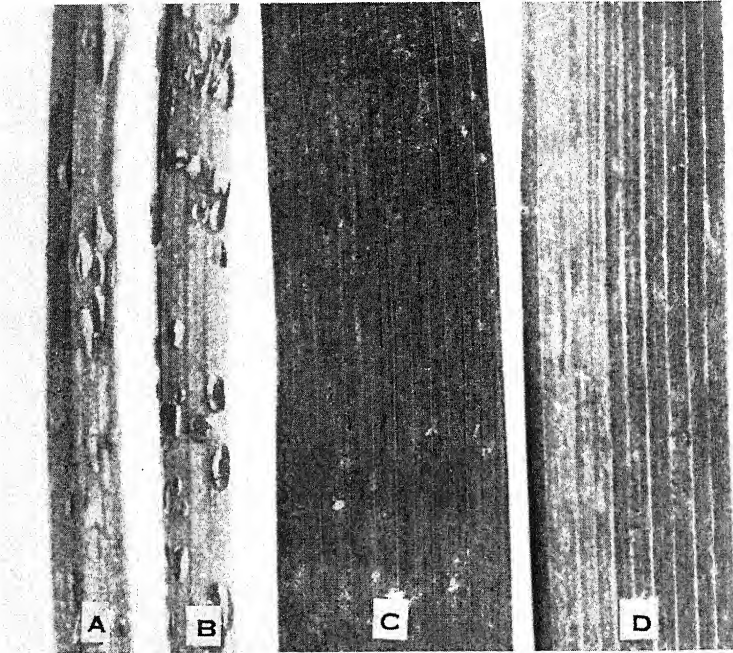


Fig. 2. Reaction to *Puccinia Iridis* sp. f. *australis*. A. *Hermodactylus tuberosus*, very susceptible. B. *Iris Xiphium*, very susceptible. C. *Iris foetidissima* (149), highly resistant. D. *Iris spuria* (*ochroleuca* 158), highly resistant. (About natural size.)

Ker-Gawl., *I. ensata* Thunb., *I. missouriensis* Nutt., *I. setosa* Pallas, *I. verna* L.

Pardanthopsis section: *Iris dichotoma* Pallas.

Evansia section: *Iris tectorum* Maxim., *I. cristata* Solander (*I. lacustris* Nutt.).

Oncocyclus section: *Iris susiana* L. Also a hybrid with an iris of the Regelia section under the name of *I. regelio-cyclus*.

Pogoniris section: *Iris pumila* L., *I. variegata* L., *I. Kochii* A. Kerner, *I. mesopotamica* Dykes. There is considerable confusion in regard to the spe-

² A study by E. Anderson (Ann. Missouri Bot. Garden 15: 241-332. 1928) indicates that this is probably a distinct species.

cies in this section. Among the varieties of bearded iris studied, such types as *I. florentina* Lam., *I. germanica* L., *I. pallida* Lam., *I. flavescens* DC., and *I. trojana* Kerner were represented.

Xiphium section: *Iris xiphioides* Ehrh., *I. Xiphium* L., and *I. filifolia* Boiss.

In a number of species, plants were obtained from several different sources. For this reason, in giving the results, the species is usually fol-

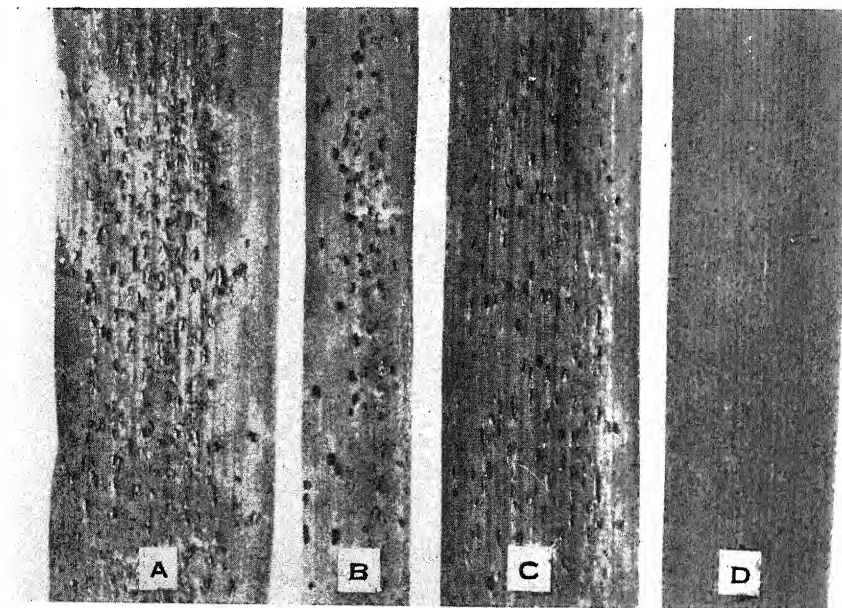


Fig. 3. Reaction to *Puccinia Iridis* sp. f. *septentrinalis*. A. *Iris foliosa* (165), susceptible. B. *Iris fulva* (32), susceptible. C. Dorothea K. Williamson (G 3) (*I. fulva* \times *I. foliosa*), susceptible. D. Dorothea K. Williamson (110) (*I. fulva* \times *I. foliosa*), highly resistant. (Reduced.)

lowed by the accession number or in some cases varietal name of the particular strain employed. The varieties of the bearded iris are mostly listed under their horticultural names.

For the most part the inoculations were made in the greenhouse under temperatures and conditions of humidity favorable for spore germination and infection. In the garden, notes were taken only after repeated exposures to infection by dusting with spores and exposure to natural inoculation from rusted species.

RESULTS WITH CULTURE I I

This culture of rust was obtained from rusted *Iris fulva* at Lafayette, Indiana, and was studied in the greenhouse during November, February, and April. The following proved to be very susceptible: *Iris foliosa* (165),

fig. 3A; *I. fulva* (32), fig. 3B; *I. fulva* (164); *I. missouriensis* (175), fig. 1A; *I. regelio-cyclus*; *I. spuria* (128); *I. spuria* (*desertorum* X 143); *I. susiana*; *I. virginica* (C2); *I. xiphioides* (varieties Prince of Wales, fig. 1B, and Royal Blue); *I. Xiphium*, fig. 2B (varieties Cajanus and Reconnaissance); and *Hermodactylus tuberosus* (*I. tuberosa*), fig. 2A. *Iris dichotoma* (100) gave a somewhat variable reaction. One inoculation resulted in a moderate production of well-developed uredinia and was classed as moderately susceptible. Another resulted in the development of considerable chlorosis and necrosis surrounding the uredinia and was classed as moderately resistant. *Iris filifolia* (variety Hort Nibbrig), *I. spuria* (*ochroleuca* X 75), and *I. virginica* (C3) were moderately resistant. *I. Douglasiana*, fig. 1C, *I. foetidissima* (149), fig. 2C, *I. graminea* (X 91), fig. 1D, *I. pseudacorus* (33), *I. sibirica* (X 94), *I. spuria* (*aurea* 157), *I. spuria* (*ochroleuca* 158), fig. 2D, *I. tectorum* (X 76), *I. tenax*, *I. verna* (169), and *I. virginica* (C. L.) were extremely resistant, showing only slight flecks or exhibiting no signs of infection. The varieties Carthusian, Fairy, Ma Mie, and Pallida Dalmatica, horticultural varieties of bearded iris, were also extremely resistant.

A study of the variety Dorothea K. Williamson proved to be very interesting. Plants from the stock (110) were very resistant to I 1, fig. 3D. Plants of the same variety obtained from a planting at Remington, Indiana, showed marked differences in reaction. Plants G2 and G3, fig. 3C, were very susceptible, while G1 was highly resistant, showing only a slight necrosis. As a result of an inquiry concerning the history of the variety, Mr. E. B. Williamson stated that it was obtained by pollinating *Iris fulva* by *I. foliosa*. About 300 plants were raised from the seed thus produced. These showed considerable variation in color from very red through various gradations to intense purple. Mr. Williamson believed that he discarded all except one of the most intense purples which he named Dorothea K. Williamson. The discards were given to a number of people. Supposedly the variety has been vegetatively propagated from one plant and should, therefore, be uniform in its rust reaction. It is possible that the differences noted may have developed through bud sports. Mr. Williamson had suggested that some of those receiving discarded seedlings may have noted the close similarity of some of the intense purples to Dorothea K. Williamson and may have propagated such under that name. The variety also seeds fairly abundantly, and some of the seedlings may have become mixed with the variety.

Culture I 1 of the rust was used to inoculate a number of species and varieties in the writer's garden during the summer of 1928. *Iris Clarkei* (X 166), *I. foliosa* (165), *I. fulva* (32 and 164), *I. missouriensis* (*tolmieana* 150), *I. setosa* (X 69), *I. Sintenisii* (162), *I. spuria* (*halophila* 99), and *I. spuria* (123) were very susceptible. *Iris Bulleyana* (X 81), *I. foliosa* (155), *I. setosa* (X 47), *I. spuria* (*halophila* X 89), *I. spuria* (X 44, 163, 128, 121, and 122), and *I. virginica* (X 92, 130) were moderately resistant. *Iris cristata* (55), *I. cristata* (*lacustris* 125), *I. dichotoma* (100), *I. ensata* (X 78, 98,

X 83), *I. graminea* (126, 167), *I. hexagona* (156), *I. Kaempferi*, *I. Kochii* (137), *I. mesopotamica* (115), *I. orientalis* (X 167, X 188, 42), *I. pseudacorus* (129, X 93, X 70, 33), *I. pumila* (31, 56), *I. sibirica* (X 94, X 82, 95, 30), *I. spuria* (*aurea* 157), *I. spuria* (*halophila* 127), *I. spuria* (*ochroleuca* 166, 158, 118), *I. tectorum* (X 76), *I. unguicularis* (*stylosa* 111), *I. variegata* (X 80), and *I. verna* (169) were highly resistant. The beardless variety Dorothea K. Williamson (110) showed only brownish spots.

The following bearded iris varieties were highly resistant, showing no signs of infection although repeatedly inoculated: Afterglow, Albert Victor, Alcazar, Anna Farr, Aurea, Ballerine, Blue Boy, Blue Jay, Caprice, Carthusian, Celeste, Dalila, Dr. Bernice, Fairy, Flavescens, Florentina, Georgia, Gertrude, Gypsy Queen, Her Majesty, Honorabile, Iris King, Isoline, James Boyd, Jacquesiana, Juniata, Lent A. Williamson, Lohengrin, Loreley, Mary Garden, May Queen, Mme. Chereau, Mithras, Monsignor, Mrs. H. Darwin, Mrs. Neubronner, Nine Wells, Oriflamme, Othello, Pallida Dalmatica, Parc de Neuilly, Parisiana, Perfection, Powhatan, Purple King, Quaker Lady, Queen Alexandria, Queen Caterina, Rhein Nixe, Sarpedon, Sherwin Wright, Storm Cloud, Violacea Grandiflora, Virginia Moore, and White Knight.

In only one instance did teliospores develop. These occurred abundantly on *Iris Clarkei* in the autumn. Tranzschel (1923) has reported connecting the rust of *Iris* with aecia on *Valeriana*. He very kindly furnished seed of several species of *Valeriana*, and plants were placed beside the over-wintered telia in the spring of 1930. However, no infection resulted. It is doubtful whether these negative results are of any significance, since tests of the teliospores did not show germination.

RESULTS WITH CULTURE I 2

In July, 1928, a collection of rust on the variety Dorothea K. Williamson obtained at Remington, Indiana, was received from Mr. H. F. Dietz. This collection (I 2) was especially interesting, since Dorothea K. Williamson (110) had previously been very resistant to culture I 1. The rust was propagated on the rusted plants obtained from Remington, Indiana, and was studied in the greenhouse during the winter of 1928-1929.

The species *Iris missouriensis* (175), *I. spuria* (*desertorum* X 143), *I. xiphoides* (variety Royal Blue), and *Hermodactylus tuberosus* were very susceptible. *Iris fulva* (32), fig. 4B, was very resistant, showing only slight necrosis or occasionally a few small uredinia. *Iris pseudacorus* (33), *I. spuria* (*aurea* 157), *I. tectorum* (X 76), and *I. virginica* (C. L.) were highly resistant, showing no signs of infection.

The variety Dorothea K. Williamson showed similar reactions as given under I 1. The strains 110 and G 1 were very resistant, while the strains G 2 and G 3 were susceptible.

Culture I 2 showed one important difference from I 1. *Iris fulva* (32), which was very susceptible to I 1, was very resistant to I 2, showing considerable necrosis and developing only a few small uredinia, fig. 4.

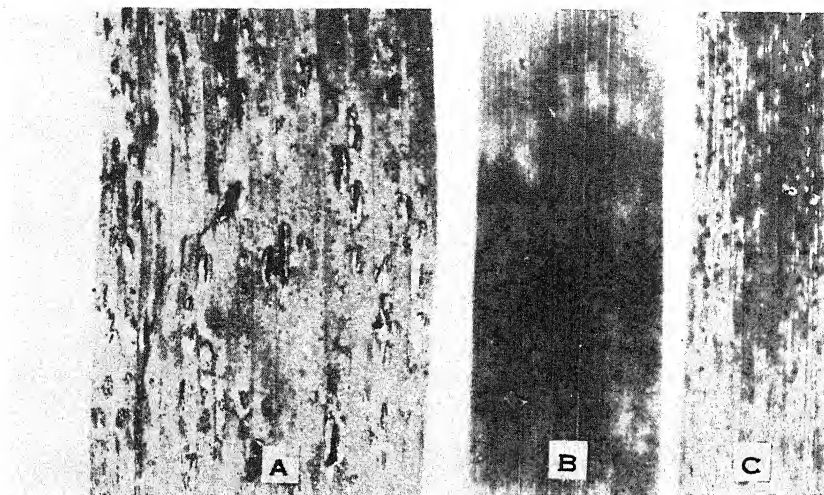


Fig. 4. Reaction of *Iris fulva* to races of *Puccinia Iridis*. A. To race *australis*, very susceptible, culture I 1 from *I. fulva*, West Lafayette, Indiana. B and C. To race *septentrionalis*. B. Culture I 2 from Dorothea K. Williamson, Remington, Indiana. C. Culture I 3 from *Iris virginica*, Coldwater, Michigan. (Somewhat enlarged.)

RESULTS WITH CULTURE I 3

During the summer of 1928, the writer collected rust on plants of *Iris virginica* at Coldwater, Michigan. At the time of collection, it was noted that several plants were heavily rusted, while adjacent plants were free. Both rusted and rust-free plants were collected and grown in the greenhouse. The rust was propagated on the rusted plants and studied during the winter of 1928-1929.

The species *Iris dichotoma* (100), *I. regelio-cyclus*, *I. xiphioides* (varieties Prince of Wales and Royal Blue), *I. Xiphium* (variety Cajanus), and *Hermodactylus tuberosus* were very susceptible.

Iris virginica showed very interesting differences. The rusted plants (C 1 and C 2) upon which the rust was collected continued to be very susceptible. Of the adjacent rust-free plants, one (C 3) was moderately susceptible, developing fewer and smaller uredinia than C 1 and C 2. Two plants (C 4 and C 5) were very resistant, developing only a few small uredinia accompanied by necrosis. Two other collections (CR and CL) of *Iris virginica* were also very resistant.

The various strains of Dorothea K. Williamson gave similar reactions to those given to cultures I 1 and I 2, strain G 2 being very susceptible, while strains 110 and G 1 were very resistant.

Iris fulva (32), fig. 4 C, showed considerable resistance, developing a moderate number of small uredinia accompanied by necrosis. *Iris fulva* (164) was more resistant, only a few very small uredinia accompanied by necrosis being produced.

Iris Douglasiana, *I. foetidissima* (141), *I. foliosa* (165), *I. pseudacorus* (33), *I. sibirica* (X 94), *I. spuria* (*ochroleuca* 158), and *I. tectorum* (X 76) were highly resistant, only occasionally showing slight flecking. The varieties, Carthusian, Fairy, Ma Mie, and Pallida Dalmatica of the bearded iris, showed no signs of infection.

During the summer of 1929 this culture was used to inoculate a series of species and varieties of iris in the writer's garden, since the rust from culture I 1 used the previous summer had not survived. *Iris missouriensis* (150), *I. setosa* (X 69), *I. spuria* (*halophila* 99), and *I. xiphioides* (Royal Blue) were severely rusted. Of a number of seedlings of *Iris spuria* (X 77), one was very susceptible, while a number were resistant, showing only a trace of rust. *Iris Bulleyana* (X 91), *I. cristata* (*lacustris* 125), *I. dichotoma* (100), *I. setosa* (X 47), and *I. virginica* (X 92) were moderately resistant. *Iris ensata* (98, X 78, X 83), *I. fulva* (32), *I. graminea* (126), *I. Kaempferi*, *I. orientalis* (142, X 188), *I. pseudacorus* (X 93, X 70, 33), *I. sibirica* (30, 95, X 82), *I. tectorum* (X 76), *I. unguicularis* (*stylosa* 111), and *I. virginica* (54) were highly resistant, either showing no signs of infection or only slight brown flecks.

The beardless variety, Dorothea K. Williamson (110), showed only slight brown flecks. The following varieties of bearded iris showed no signs of infection: Albert Victor, Aurea, Ballerine, Blue Boy, Blue Jay, Caprice, Carthusian, Celeste, Dr. Bernice, Fairy, Flavescens, Florentina, Georgia, Gypsy Queen, Her Majesty, Honorabile, Iris King, James Boyd, Jacquesiana, Lent A. Williamson, Lohengrin, Loreley, Mary Garden, May Queen, Mme. Chereau, Mithras, Monsignor, Mrs. H. Darwin, Mrs. Neubronner, Nine Wells, Pallida Dalmatica, Parisiana, Parc de Neuilly, Perfection, Purple King, Quaker Lady, Queen Alexandria, Queen Caterina, Rhine Nixe, Sherwin Wright, Storm Cloud, Violaacea Grandiflora, and White Knight.

RESULTS WITH CULTURE I 4

This culture was obtained from *Iris spuria* in the Botanical Garden of the University of Michigan, Ann Arbor, Michigan, in the summer of 1931. Rusted plants were transplanted to the greenhouse of the Botanical Garden in the autumn and the rust was studied during the winter of 1931-1932. *Iris dichotoma* (BG 4862), *I. spuria* (BG 12685, BG 9166, BG 6752), *I. versicolor* (BG 13219), and *I. Xiphium* were susceptible.

Iris spuria (BG 7097) was moderately resistant. *Iris fulva* (32 and 164) was resistant, showing only small uredinia accompanied by necrosis. *Iris Douglasiana* (1560), *I. ensata* (BG 10697), *I. graminea* (X 91 and BG 7069), *I. mesopotamica* (115), *I. pumila* (BG 5531), *I. Purdyii* (1558), *I. sibirica* (BG 6703), *I. spuria* (BG 12683), *I. tenax* (1559), *I. verna* (169), and the variety Dorothea K. Williamson (110) were highly resistant. The group here listed under *I. spuria* contains a number of intergrading forms, many of which have been recognized as species by some botanists. It is

evident that the group also shows considerable variation in its rust reaction. *Iris tectorum* (X 76) produced a few well-developed uredinia as the result of one inoculation. In other tests only somewhat indistinct chlorotic spots were developed. The bearded iris varieties, Afterglow, Alcazar, Fairy, George Tribolet, Mrs. Neubronner, and Sherwin Wright, showed no evidence of infection following inoculation.

This culture agrees very well with cultures I 2 and I 3, especially in the resistance of *Iris fulva*, and differs in this respect from culture I 1 to which that species was susceptible.

SUMMARY AND CONCLUSIONS

From results which have been given it is evident that there are at least two races of *Puccinia Iridis*. These may be designated as follows:

Puccinia Iridis sp. f. *australis*

This race is distinguished by the marked susceptibility of *Iris fulva* (32 and 164), fig. 4A, and *Iris foliosa* (165). It was obtained from *Iris fulva* at West Lafayette, Indiana.

Puccinia Iridis sp. f. *septentrionalis*

This race is distinguished by the marked resistance of *Iris fulva* (32 and 164), fig. 4B, C, and *Iris foliosa* (165). It was collected on *Iris virginica* at Coldwater, Michigan, on *Iris spuria* at Ann Arbor, Michigan, and on the variety Dorothea K. Williamson (*I. fulva* × *I. foliosa*) at Remington, Indiana.

Other than the differences exhibited by the reactions of *Iris fulva* and *I. foliosa*, the two races produced very similar reactions on the species and varieties investigated. According to the classification of Dykes (1913), species were studied in the sections Apogon, Pardanthopsis, Evansia, Pogoniris, and Xiphium. The collections of rust were all obtained from species in the section Apogon.

In the sibirica group of the Apogon section, *Iris sibirica* and *I. orientalis* were highly resistant. *I. Bulleyana* showed moderate resistance and *I. Clarkei* was very susceptible. Abundant telia developed on the last species.

In the spuria group of the Apogon section, *Iris graminea* was highly resistant. *Iris spuria* showed considerable variation in reaction. Very susceptible, moderately resistant, and highly resistant strains were found. This species is apparently very variable and contains a number of forms which have been considered distinct species by some authors. There is considerable intergradation and many of the forms and subspecies cannot be separated with certainty. As far as studied, the subspecies *aurea* and *ochroleuca* were more or less resistant. The variety *halophila* was found to contain both resistant and susceptible individuals. The Sydows (1904) list both *Iris graminea* and *I. ochroleuca* as hosts for the rust.

In the California group of the Apogon section the three species studied, *Iris Douglasiana*, *I. tenax*, and *I. Purdyi*, were all highly resistant. The Sydows (1904) give *I. Douglasiana* and Arthur (1920) *I. Douglasiana* and *I. tenax* as favorable hosts for the rust.

In the scarlet-seeded group of the Apogon section, the only species, *Iris foetidissima*, was highly resistant. The Sydows (1904) list this as a host for the rust.

In the unguicularis group of the Apogon section, the only species, *Iris unguicularis*, was extremely resistant.

In the laevigata group of the Apogon section, *Iris Kaempferi* and *I. pseudacorus* were extremely resistant. *Iris virginica* showed considerable variation, some plants being very susceptible, others showing various degrees of resistance, and some being very resistant. The Sydows (1904) list *I. pseudacorus* as a host.

In the hexagona group of the Apogon section *Iris fulva* and one strain of *I. foliosa* (165) were susceptible to the race *australis*, resistant to the race *septentrionalis*. Another strain (155) of *Iris foliosa* was moderately resistant to race *australis*.

In the ensata group of the Apogon section the only species, *Iris ensata*, was extremely resistant. The Sydows list this as a host.

In the longipetala group of the Apogon section only *Iris missouriensis* was studied. This was susceptible.

In the tripetalous group of the Apogon section only the species *Iris setosa* was studied. This showed considerable variation, some plants being susceptible and others moderately resistant.

In the verna group of the Apogon section the only species, *Iris verna*, was extremely resistant.

In the Pardanthopsis section of the genus, *Iris dichotoma* sometimes gave a susceptible type of reaction and at others showed more or less resistance.

In the Evansia section, *Iris cristata* was extremely resistant. *Iris cristata* (*lacustris*) was moderately resistant. *Iris tectorum* was usually resistant, although occasionally it produced a moderate number of well-developed uredinia. The Sydows list the latter as a host.

In the Onocyclus section the only species studied, *Iris susiana*, was very susceptible.

All the iris studied of the Pogoniris section were extremely resistant. Included were *Iris Kochii*, *I. mesopotamica*, *I. pumila*, *I. flavescens*, *I. variegata*, and *I. pallida*, as well as many horticultural varieties which, according to Dykes, were probably derived largely from hybrids between *I. pallida* and *I. variegata*. The Sydows list *I. flavescens*, *I. pumila*, and *I. pallida* as hosts. They also list several other species of this group. So far as the writer is aware, rust has apparently only been found once on bearded iris in the United States. W. N. Shear in a letter stated that rust was collected on German iris in 1928 in the vicinity of San Diego, California, but that special pains were

taken to destroy all infected plants and he was not able to find any rust on this group of iris the next year.

In the Xiphium section the varieties studied of *Iris Xiphium* and *I. xiphoides* were very susceptible. *Iris filifolia* was more or less resistant.

The marked susceptibility of *Hermodactylus tuberosus* is of special interest, since it gives a very susceptible species outside of the genus *Iris*, although it has been included by some under the name *Iris tuberosa*.

It is evident that as far as the two races studied of *Puccinia Iridis* are concerned, there is no correlation between rust reaction and relationship of the species in the genus. Susceptible species were found in such dissimilar sections as Apogon, Onocyclus, Xiphium, and in the genus *Hermodactylus*, while other species of the sections Apogon and Xiphium showed no signs of infection.

The differences noted between the host range for the rust given by the Sydows and Arthur as contrasted with the susceptible species determined in this study indicate that probably several other races may be distinguished in the species. Part of this discrepancy may possibly be attributed to the strains of the species studied, since, as shown for *Iris spuria* and *I. virginica*, different strains of a species may give markedly different reactions. This can hardly explain all of the differences. The marked resistance in these studies of all the Pogoniris section and the Californian group of the Apogon section indicates that there are probably at least two other races differentiated by their specialization to these groups. The marked resistance of such species as *Iris foetidissima*, *I. pseudacorus*, *I. ensata*, and *I. sibirica* as contrasted with the susceptibility reported by Plowright, the Sydows, and Tranzschel indicates that there may also be races specialized to these species.

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THE PRODUCTION OF TIMOTHY POLLEN ¹

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The pollen of timothy (*Phleum pratense*), like the pollen of many other grasses and of other kinds of plants, is capable of producing hay fever in persons susceptible to it (Bernton, Jones, and Csonka, 1927). Within recent years medical investigators and practitioners have discovered that if the particular kind of pollen which produces the hay fever symptoms in any particular patient be determined, in a large proportion of the cases partial or complete relief may be obtained, temporarily, by treating the patient with extracts prepared from the proper pollen (Duke, 1925, p. 242).

The development of a use for timothy pollen, for the preparation of pollen extracts, has created a number of new problems, some of which have been studied at the Timothy Breeding Station, which is located in the northern part of Ohio. After giving a brief description of the flowering habits of timothy and of methods which may be used in collecting the pollen, this paper presents a more detailed account of the following investigations of problems associated with the production of timothy pollen which have been conducted here: (1) the effect of nitrate of soda, applied to the soil, upon the yields of pollen; (2) the relation of weather conditions to the daily yields of pollen; and (3) the seasons during which early, medium, and late varieties of timothy produce pollen.

FLOWERING HABIT OF TIMOTHY

In the latitude of northern Ohio, florets bloom on plants of ordinary timothy in the largest numbers during the latter part of June and the early part of July. The flowering process may extend over a period of nearly a month, but the time during which very large numbers of florets are in bloom is restricted to about 10 days. This period of maximum bloom, in meadows of ordinary timothy, in most seasons extends from about June 25 to July 5—the actual dates in some years being slightly earlier or later, depending upon seasonal weather conditions (Evans, 1927, p. 38-41). South of northern Ohio timothy blooms somewhat earlier, while north of this latitude it blooms a little later than the dates which have been indicated.

The florets of timothy bloom at a fairly definite time during the day. Usually the largest numbers of florets come into bloom at about sunrise,

¹ Contribution from the Timothy Breeding Station, North Ridgeville, Ohio, which is maintained coöperatively by the Office of Forage Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, and the Department of Agronomy, Ohio Agricultural Experiment Station.

though some of them may bloom soon after midnight, or even 2 or 3 hours after sunrise.

The flowering process in timothy is easily affected by weather conditions. If the conditions are unfavorable, no florets may bloom. This phase of the flowering habits of timothy is discussed more fully in the paragraphs under the heading "Relation of weather conditions to yields of pollen."

When the anther sac in which the pollen is contained first emerges from within the scales of the timothy floret, its walls are intact, confining the pollen grains within it. For a time there is no opportunity for the pollen to escape. As the anther becomes dry, it opens longitudinally—or dehisces. Its walls may split apart suddenly, so that the pollen is expelled in the form of a miniature cloud. Or, especially if there is no wind in the early morning, the anthers may gradually dehisce on very large numbers of florets, with but little dispersion of pollen. If a breeze then suddenly arises and passes over the meadow, the stems are shaken and the pollen is released. Under such conditions it occasionally forms a hazy cloud over the field, which may be seen from a long distance.

METHODS OF COLLECTING POLLEN

One may collect timothy pollen in the field by shaking the heads over a pan or other receptacle. This method, however, is a slow one, and the presence of dew on the timothy heads at the time when the pollen is escaping usually makes it difficult to obtain the pollen in a satisfactory condition.

Kelly (1928) has devised a method of gathering pollen of different kinds of plants. The bases of the stems are placed in jars containing water, while the upper parts of the stems, where the florets are located, are over sheets of paper on which the pollen is collected.

At the Timothy Breeding Station, when timothy pollen is to be collected, the stems are cut, at full length, during the latter part of the afternoon. They are then taken to a building with a suitable floor, and are spread in swaths which are sometimes made 3 or 4 inches thick. The upper parts of the stems with the heads are placed over long sheets of wrapping paper, which are 24 to 30 inches in width. Sometimes it has been the practice—especially if the temperature is comparatively high—to place burlap partially saturated with water over the bases of the stems to prevent evaporation; however, the paper on which the pollen is to be collected must be kept dry.

When the timothy stems are brought to a building and handled in this way, the florets bloom on the following morning, in much the same way as though the stems had been left uncut in the field. During the latter part of the forenoon the heads are gently shaken over the sheets of paper, so that the pollen falls on them; the stems are then discarded. If no florets bloom on the first morning, they may be kept, and more or less pollen obtained on the second morning.

The pollen must be dried carefully, or else it may be damaged in the process. After it is thoroughly dry, it may be separated from anthers and other

foreign material by means of a fine-meshed metal sieve—such as is used in making milk strainers.

Pollen of orchard grass (*Dactylis glomerata*) and of Kentucky bluegrass (*Poa pratensis*) has been collected by essentially the same methods which have been described for timothy. The yields of orchard grass pollen are about equal to those of timothy, but the yields obtained of the pollen of Kentucky bluegrass have been relatively small.

EFFECTS OF APPLICATIONS OF NITRATE OF SODA ON YIELDS OF POLLEN

In two seasons, the effects of a nitrogen-carrying fertilizer upon the yields of timothy pollen have been determined. In each year, nitrate of soda was applied on the soil in alternate plats at the rate of 160 pounds per acre. In 1926 this fertilizer was applied, on May 6, on plats which measured 61×8.25 feet; in 1927 the nitrate was applied, on May 20, on plats measuring 33×8.25 feet. In both years the plats were located in a meadow of Huron timothy, which is a variety which blooms about 6 days later than ordinary timothy. On each date when timothy was collected, the crop on 2 unfertilized and on 2 fertilized plats was harvested; the duplicate plats were distributed in different parts of the series. The date on which the collection of pollen from each set of plats is recorded is on the day following the one on which the timothy stems were harvested in the meadow.

In both years hay, also, was harvested from a number of plats in these series, and its air-dry weight determined.² In 1926, 4 plats, and in 1927, 8 plats, distributed through different parts of the same series of plats from which pollen was gathered, were used for hay production. From the data obtained, the relations of pollen yield to hay yield are evident.

The yields of pollen obtained on each date in 1926 are presented in table 1. In this year the first collection of pollen was made on July 7, which

TABLE 1. *Yields per acre of timothy pollen collected from unfertilized plats and from plats fertilized with nitrate of soda, on different dates in 1926*

Date of collection of pollen	Yield, pounds per acre of pollen from	
	Unfertilized plats	Fertilized plats
July 7	2.81	8.44
" 8	2.38	4.52
" 9	0.30	0.70
" 10	0.54	0.73
" 11	1.84	2.92
" 13	1.65	2.73
" 15	1.89	2.16
" 16	0.43	0.35
Average	1.48	2.82
Average percentage of increase in yield of pollen in fertilized plats		90.5

² The air-dry weight of the hay produced by each plat was obtained from typical samples collected and weighed when the entire crop was weighed in the field, and which were again weighed after the samples had been stored in the loft of a building until air dry—i.e., until they had ceased to lose weight.

is the date of maximum bloom; some pollen could have been obtained a few days earlier. The records obtained in 1927 are presented in table 2 and in the graph shown in figure 1.

TABLE 2. *Yield per acre of timothy pollen collected from unfertilized plats and from plats fertilized with nitrate of soda, on different dates in 1927*

Date of collection of pollen	Yield, pounds per acre, of pollen from	
	Unfertilized plats	Fertilized plats
July 6	0.40	0.87
" 7	0.85	1.67
" 8	0.57	1.05
" 9	2.25	4.77
" 10	0.80	1.40
" 11	0.87	1.35
" 12	0.92	2.70
" 13	0.25	0.70
" 14	0.25	0.77
" 15	0.37	0.65
" 16	0.97	1.22
" 17	0.00	0.00
Average	0.71	1.43
Average percentage of increase in yield of pollen in fertilized plats		101.4

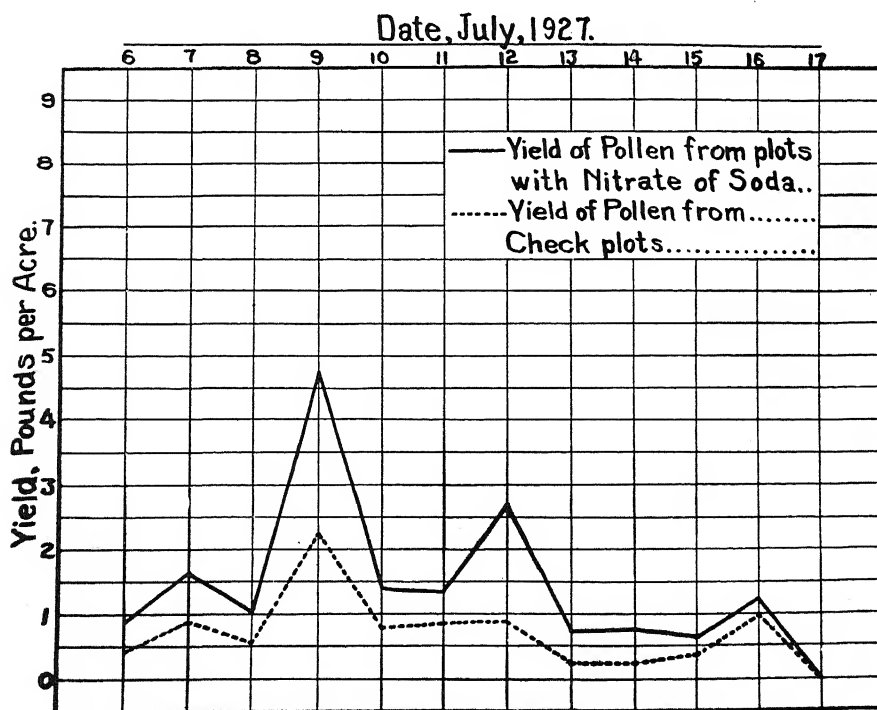


Fig. 1. Yields of pollen from unfertilized plats of timothy and from plats fertilized with nitrate of soda.

The yields of hay in plats where no fertilizer was used and from plats on which nitrate of soda was applied, in 1926 and 1927, are presented in table 3.

TABLE 3. *Yields of timothy hay on unfertilized plats and on plats fertilized with nitrate of soda at the rate of 160 pounds per acre, in 1926 and in 1927*

Year	Pounds of air-dry hay per acre in plats		Percentage increase in yield in fertilized plats
	Not fertilized	Fertilized	
1926	3099	4344	40.2
1927	2281	3279	43.8

In both years the largest yields of pollen were produced by those plats producing the largest yields of hay. The larger yields of pollen in 1926 than in 1927 may to some extent be correlated with the larger hay yields in that year.

In both 1926 and 1927 the percentages of increase in the yields of pollen, resulting from the applications of nitrate of soda, were very much greater than the percentages of increase in the yields of hay. It has been found that applications of nitrate of soda on timothy meadows result in an increased number and proportion of timothy stems producing inflorescences (Evans, 1927, p. 15-16). The greater increases in the yields of pollen than of hay on the fertilized plats may be attributed, in part at least, to the larger proportions of timothy stems on which inflorescences developed.

Another reason for the larger average daily yields of pollen in 1926 is the fact that pollen was collected on only 8 days; none was collected on the first and last dates on which collections were made in 1927, when the yields were relatively low; nor was any pollen collected on July 12 and 14, 1926, when, according to weather records, conditions were unfavorable for the blooming of timothy florets. If collections had been made on every day from July 6 to 17 in 1926, as in 1927, the average daily yields of pollen in these two seasons probably would have more nearly corresponded with the relative hay yields.

RELATION OF WEATHER CONDITIONS TO YIELDS OF POLLEN

In table 4 records are shown of the yields of timothy pollen, harvested in 1926, from plats of Huron timothy which had been fertilized in the spring with nitrate of soda. Data in regard to the maximum and minimum temperature, the amount of rainfall, and the per cent of sunshine on each date are also presented. No pollen was collected on those dates for which there are no records in the column under "yield of pollen."

An examination of this table and of similar records for 1927 and 1928 shows that the largest yields of pollen were usually harvested on days accompanied or preceded by a minimum temperature of about 60 degrees Fahrenheit or higher, with an absence of rainfall and a comparatively high percentage

of sunshine. Occasionally good yields may be obtained when one or more of these favorable conditions do not exist; this is most likely to occur after the flowering process has already been suppressed by unfavorable weather on one or more preceding days.

TABLE 4. *Relation of weather conditions to the yields of timothy pollen. ("T" indicates only a trace of rainfall—too small to measure)*

Date		Weather conditions				Yield of pollen, pounds per acre	
		Temperature		Pre- cipitation, inches	Per cent sunshine		
		Maximum	Minimum				
1926							
July	6	83	69	T	71	—
"	7	77	65	0.00	99	8.44
"	8	89	63	0.00	97	4.52
"	9	90	74	0.18	79	0.70
"	10	80	62	0.58	23	0.73
"	11	72	59	0.00	100	2.92
"	12	77	59	0.14	55	—
"	13	64	56	0.74	45	2.73
"	14	69	51	T	89	—
"	15	72	55	0.00	97	2.16
"	16	81	58	0.00	100	0.35

If the temperature is about normal or above normal and the sky remains clear, the flowering process continues without interruption at the usual time each day, on any timothy plant or in any timothy meadow, until completed.

If the weather becomes cloudy, and especially if there is a rainfall and the temperature becomes subnormal, timothy florets are likely to cease blooming partially or entirely for one or even for two days. It has been observed that if the minimum temperature becomes as low as 50° or 52° Fahrenheit, even though the sky be clear, timothy florets are not likely to bloom.

On the day following such unfavorable weather, especially if conditions have become very favorable again, the florets bloom in exceedingly large numbers. Apparently those florets in which the flowering process had been suppressed for one or two days bloom simultaneously with those which would normally bloom on the day when the favorable conditions again occur.

SEASON DURING WHICH DIFFERENT VARIETIES OF TIMOTHY PRODUCE POLLEN

The time in any season during which timothy pollen may be collected may be extended by growing varieties which bloom early, medium, and late. An experiment was conducted in 1928, the results of which show how the season for the collection of pollen may be lengthened in this way.

Two series of plats, one in a meadow of ordinary timothy which blooms at a medium time, and one in a meadow of Huron timothy—which is a late variety—were used in this experiment. Each plat of ordinary timothy measured 49.5 × 4.125 feet, and each plat of Huron timothy measured 44.0 × 8.25 feet. Timothy stems were harvested from two plats located in

different parts of each series, on each date of collection. The yields obtained are recorded in figure 2.

Ordinary timothy produced yields of pollen exceeding 2.5 pounds per acre on five dates—July 1, 2, 3, 6, and 7; Huron timothy produced yields exceeding 2.5 pounds per acre on only three dates—July 6, 7, and 11, but the yield of pollen was larger from the plats of Huron timothy on every one of these three dates than it was on any date in the plats of ordinary timothy. Because the different varieties were growing in different locations, and since

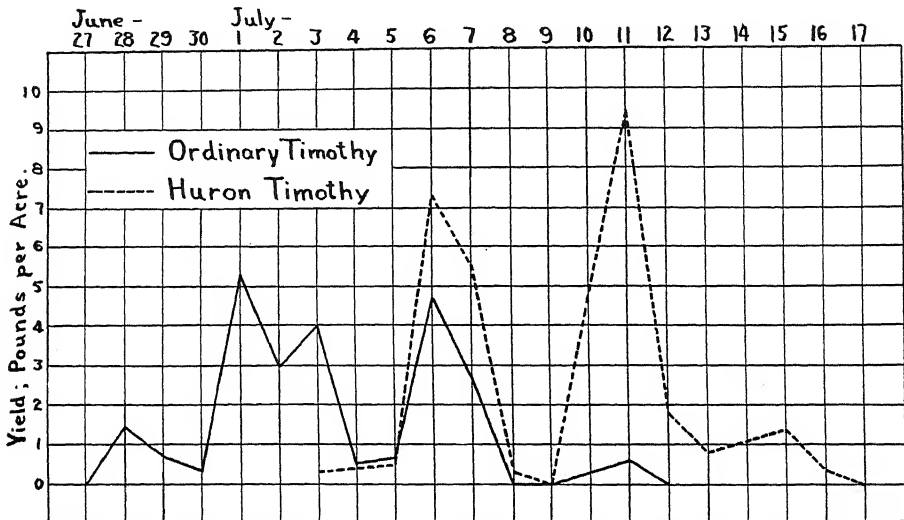


Fig. 2. Comparison of yields of pollen produced by ordinary and Huron timothy on different dates in 1928.

the pollen was harvested from them on different dates and under different weather conditions, an accurate comparison of the relative yields produced by the two varieties is hardly possible. The data obtained do show, however, how the season for the collection of pollen may be extended by harvesting from varieties of timothy blooming at different times. There are early varieties of timothy which bloom approximately as much earlier than ordinary timothy as Huron timothy is later than the ordinary variety.

SUMMARY

Extracts of timothy pollen are used for the treatment of hay fever in patients who are susceptible to the effects of the pollen of this species.

In the latitude of northern Ohio, timothy florets bloom in the largest numbers in meadows of ordinary timothy from about June 25 to July 5. The season is somewhat earlier south and a little later north of this latitude.

Timothy pollen may be collected by harvesting the stems on afternoons during the flowering season and placing them with the heads over sheets of

paper. On the following morning the florets usually bloom in much the same way as in the field. The pollen may then be shaken off and collected on the sheets of paper. Pollen of other grasses has been collected by essentially the same methods which have been used for timothy.

In experiments conducted in 1926 and 1927, the yields of pollen were increased as a result of applications of nitrate of soda to the soil. In each year the percentages of increase in the yields of pollen resulting from the applications of nitrate of soda were more than twice as large as the corresponding increase in yields of hay.

The largest yields of pollen are usually produced on days when the temperature is about normal or above normal, and when there is no rainfall and a relatively high percentage of sunshine. As long as favorable weather continues, the process of blooming occurs at about the same time each day during the flowering season until completed. When the weather becomes cloudy and there is rainfall, and especially if the temperature becomes sub-normal, the process of blooming may be suppressed for one or even two days. When favorable weather conditions again occur, the florets bloom in unusually large numbers.

By growing early and late varieties, the time during which timothy pollen may be collected may be extended about a week earlier and a week later than for ordinary timothy.

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CONCENTRATION OF THE VIRUS OF THE MOSAIC OF TOBACCO

BURT JOHNSON

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A number of attempts have been made to purify several of the viruses causing diseases of plants. Alcohol, acetone, charcoal, talc, and a number of other substances have been used to separate viruses from the complex of substances in the juices of diseased plants. Owing to the fact that the only known measurement for the concentration of virus is the biological one, the principal problem in such precipitations and adsorptions is to free the virus from the other precipitated materials, or to remove the virus from the adsorbing agent, as the case may be, without inactivating the virus. In the papers mentioned below, the earlier fundamental experiments on these processes have been reviewed and need not again be cited here.

Brewer, Kraybill, Samson, and Gardner (1930) employed a supercentrifuge to make the initial separation of the virus from the bulk of the liquid of the juice of the plant. The resulting gummy residue containing the virus was resuspended and again centrifuged, this time the virus remaining in suspension. The suspension was further cleared by the addition of alumina gel having an acid reaction. A clear and colorless suspension of the virus of the original virulence results.

Vinson and Petre (1929, 1931) tested a number of possible methods with varying success. A good result was obtained by using Horne's basic lead acetate to precipitate the virus from the plant juice. The subsequent elution and clearing of the virus suspension was attained by washing with water and treatment with primary potassium ortho-phosphate and secondary potassium ortho-phosphate. This method gave preparations of high purity and great virulence. They have, in addition to other methods, also treated the juice of diseased tobacco plants with various salts and found that the virus was salted out with ammonium sulfate and magnesium sulfate. They discontinued the salting out methods because the precipitates "contained the excess salt, but also protein and much pigment." Vinson (1932) has recently reported a method of purification by precipitating the virus with safranin and then removing the safranin with Lloyd's alkaloidal reagent.

That there is a possibility of at least partially purifying suspensions of virus by subjecting them to electrophoresis at pH 4.0 is suggested by Takahashi and Rawlins (1930).

The purpose of the present paper is to report the results of efforts to concentrate the virus of the typical mosaic of tobacco, during which process

a certain amount of purification of the virus is achieved. It is believed that in addition to purified preparations of virus concentrated preparations are also necessary in the study of the properties of the virus.

GENERAL METHODS

In this section will be listed certain methods used throughout the experiments. Any variations in these methods will be noted in the proper places.

The variety of tobacco used was Wisconsin Havana 142. All inoculations were done by placing a loopful of the preparation being tested in the axil of a single leaf of a plant, and then this axil was pricked through the drop five or six times with a needle. A small scratch was placed on the leaf of an inoculated plant. The loop held approximately $1/140$ cubic centimeter of water.

Tobacco plants having symptoms of mosaic were ground in a food chopper and the juice from the ground material was expressed through cheesecloth. Such juice from diseased plants is designated in the rest of this paper as "1/1 diseased juice." All control inoculations were made by dilutions with water of an aliquot of this original diseased juice. "1/5 (and 1/10) Celite-treated diseased juice" was prepared by the method devised by Duggar.¹ Both "1/1 healthy juice" and "1/5 (and 1/10) Celite-treated healthy juice" were made for comparative studies just as the diseased juices had been prepared.

In each experiment ten plants were inoculated for each dilution tested. Thus most experiments required not fewer than 100 plants and often more. Most of the work was conducted in a greenhouse, but the first work was done in an "insect-proof" cheesecloth tent. The results were apparently not affected by the location.

All the collodion membranes were made by dissolving six grams of air-dried parlodion in a mixture of 50 cc. of absolute alcohol and 50 cc. of ether. All dialyses were through collodion membranes against running distilled water. This water carried a trace of chlorides.

Evaporations were effected to best advantage by placing the material to be evaporated in large watch glasses and then setting in the air current from an electric fan. This was quicker and safer (as regards temperature) than any other method attempted.

METHODS FOR THE PARTIAL CLEARING OF TOBACCO JUICES

Electrical precipitation. It was observed that in electro-dialysis there was, in addition to the usual settling out of certain materials in the tobacco juice upon standing, an increased and comparatively rapid and heavy precipitation during the dialysis. This is perhaps a similar phenomenon to that in certain suspensions where precipitation occurs when they are subjected

¹ Duggar, B. M. Proc. Soc. Exp. Biol. Med. 30: 1104-1109. 1933.

to an electrical current. It was found that alternating current was ineffective in causing a precipitation from tobacco juice. With a direct current of 110 volts precipitations occurred within a period of less than a minute to a few minutes.

Using direct current, the following type of experiment was performed. A beaker of 1/1 diseased juice was placed in a vessel containing ice water. This was to counteract, at least to a limited extent, the heating effects of the electric current and to provide for the rapid cooling of the juice when the current was discontinued. Electrodes made of sheet copper three cm. by five cm. on an edge (in some experiments zinc electrodes of the same size) were placed in the beaker of juice and the current was started. Thermometer readings were made continuously and the current was stopped before inactivating temperatures were attained. As soon as the juice had cooled to 40°-45° C., it was centrifuged and the residue discarded. A sample of the supernatant liquid was saved for inoculations at appropriate dilutions. The treatment just described was often repeated, but using the supernatant liquid, obtained from the first treatment as the basis for the second treatment. This resulted in a further clearing of the juice. Inoculations were also made with the supernatant liquid from this re-treated material.

In table I are found the results of several experiments as regards the effect of direct current on the virus. Included is an experiment in which carbon electrodes were used in the same manner as described above. Also, it

TABLE I. *The virulence of tobacco mosaic virus in the supernatant liquid after precipitations by direct current of 110 volts and centrifugation. Dilutions are calculated on the approximate basis of the original volume of diseased juice. "D.J." stands for "diseased juice."*

Dilution during treatment	Dilution for inoculation (after treatment)	Percentage diseased					
		Cu electrodes		Zn electrodes		C electrodes	
		First treatment	Second treatment	First treatment	Second treatment	First treatment	Second treatment
1/1 D.J.	1/100	*	*	*	*	†	†
"	1/1000	90	70	80	100	90	100
1/10 D.J.	1/100	70	40	60	70	70	100
"	1/1000	60	0	50	0		
		50	0	10	0		

* Control: 1/100 and 1/1000 dilutions of the original 1/1 diseased juice gave 90 per cent and 80 per cent infections respectively.

† Control: 1/100 and 1/1000 dilutions of the original 1/1 diseased juice gave 60 per cent and 40 per cent infections respectively.

will be noted that these experiments were duplicated using 1/10 diseased juice. The inactivation of the virus in these latter cases was marked in comparison to those in which 1/1 diseased juice is used. A suitable explanation for the high degree of virulence maintained by the 1/1 diseased juice has not been found.

As described, the electro-precipitation, although leaving the virus in suspension, does not completely clarify the plant juice. Efforts were made to effect further purification by treatment in a more acid medium, precipitating the copper from solution, and clearing with Horne's basic lead acetate. These efforts failed in that the virus in the supernatant liquid was inactivated, or precipitated and not recovered; and also, often many impurities, particularly products of electrolysis, still remained in the supernatant liquids.

The marked sensitiveness of diluted diseased juice to various treatments and the inactivation of the virus when exposed to a series of active chemical reagents are observations noted in many other experiments not recorded in this paper. This inactivation may in part be due to the removal of protective colloids. As will be shown later, it seems that no particular inactivation occurs when a series of neutral and inert reagents is used.

Salting out. A comparison of the effects of various reagents in the salting out of the virus was made. The method employed was to treat 100 cc. of 1/5 diseased juice with approximately enough of a particular reagent to saturate the diseased juice completely. After being well stirred, the preparations were held in the icebox for 18 hours and then centrifuged, but filtration in addition to centrifugation was necessary to separate the precipitates completely from the supernatant liquids. The filtrates were counted as a 1/5 dilution of the original diseased juice and were further diluted to 1/100 for inoculation into the test plants.

The precipitates were removed as completely as possible from the filter papers in a little water and separately dialyzed for four days. After dialysis the volume of the dialyzed material was reduced from one-fourth to one-third of the original volume of 1/1 diseased juice used. The evaporated materials were diluted for inoculations, and the dilutions were then calculated on the basis of the original volume of 1/1 diseased juice treated.

Referring to table 2, it is seen that salting out of the virus occurred in only five cases and then but partially. In three of the five cases where salting out did not occur, the failure may be attributed to the injurious effects of the salts at the concentrations used, for the filtrates also failed to show any virus present. There are indications of injury in the cases of sodium fluoride and the mixed potassium phosphates.

The results as regards sodium chloride are quite characteristic of a number of experiments in which this salt was used as a possible salting out agent. In this experiment it happens that sodium chloride forms a saturated solution, but in other experiments more dilute sodium chloride was used with results similar to those shown in table 2. In the cases of most of the other salts, the amounts used resulted in saturated solutions with a solid excess of the salt.

Ammonium sulfate is not included in this table, but it was found in a number of other trials that it precipitates most or all of the virus when it is used as about a 50 per cent solution with the virus suspension as the solvent.

TABLE 2. *Comparative effects of different reagents in salting out the virus of mosaic of tobacco. All dilutions of precipitates after dialysis and evaporation are calculated on the basis of the original volume of diseased juice used. The filtrates were all inoculated at a dilution of 1/100.*

Salt used	Filtrate	Precipitate	
	% diseased of 10 plants inoculated	Dilution for inoculation	% diseased of 10 plants inoculated
Control—1/1000 D.J.	40		
Magnesium sulfate	40	1/3000	20
Magnesium chloride	0	1/2500	0
Lithium nitrate	0	1/3000	0
Sodium chloride	90	1/3500	20
Sodium fluoride	40	1/2500	10
Sodium sulfate	70	1/3500	30
Sodium citrate	0	1/4000	0
Sodium nitrate	60	1/2500	0
Potassium sulfate	80	1/2500	10
Monobasic potassium phosphate plus dibasic potassium phosphate	20	1/3000	0

This salt will also bring down a great deal of the other materials in the plant juice along with the virus, particularly objectionable in this work being large amounts of any brown pigments that happen to be present.

Adsorption on charcoals. As indicated above, the precipitate from ammonium sulfate treatment after dialysis and reduction of volume was always very dark and quite opaque. In order to remove this objectionable pigment, several methods were attempted. Charcoal is known to adsorb, for example, virus of the tomato mosaic from suspensions of that virus (Brewer et al., 1927). It was not found practicable in the present work to adsorb pigments with activated carbons from the virus preparations resulting from the precipitation with ammonium sulfate. Nor was the practice of filtering the re-suspended precipitates through alundum cups useful in removing the pigment nor in retaining the virus in suspension.

The following experiment indicated a means of eliminating most of the objectionable coloring matter and at the same time leaving most of the virus in suspension. Three grades (2, OO, and W) of the activated carbon Nuchar were available for adsorptions. To 100 cc. of 1/10 Celite-treated diseased juice were added 2.5 grams of Nuchar 2. The mixture was well stirred and then filtered through filter paper. The filtrate was not quite as clear and colorless as water. A similar treatment with Nuchar W gave a filtrate apparently as clear and colorless as water, while a like treatment with Nuchar OO gave a filtrate not quite as clear and colorless as that of Nuchar W. Because the 2.5 per cent Nuchar W was so efficient in clarification, a 0.5 per cent Nuchar W was also used. This latter gave a clear filtrate of a light buff color.

In order to get a more accurate idea of the amounts of coloring matter still left in these dilute solutions (filtrates), each was reduced to a volume of

five cc. and any precipitate formed was removed by centrifugation. After this treatment the supernatant liquids may be described as follows: from 2.5 per cent Nuchar 2, not quite clear and colorless; from 2.5 per cent Nuchar W, apparently clear and colorless; from 2.5 per cent Nuchar OO, intermediate between that of the first two Nuchars; and from 0.5 per cent Nuchar W, brown in color and opaque. It would seem rather essential that so-called clear and colorless suspensions of virus at a dilution equal to or greater than the original volume of juice employed be reduced in volume to get an exact idea of the actual degree of clarity of the preparation. This seems to be a point not considered by some investigators.

Having obtained visual evidence of the clarification by the three carbons, their effects on the virus were next determined. For inoculations the evaporated materials were diluted 1000 and 10,000 times. This corresponds to an approximate dilution on the basis of the original 1/1 diseased juice used of about 1/350 and 1/3500 respectively. These latter dilutions are indicated in table 3. The control inoculation is an actual 1/1000 dilution of the 1/1

TABLE 3. *The comparative effects of three activated carbons on the virulence of diseased juice. All dilutions are approximate and are calculated on the basis of the original volume of diseased juice. Ten plants were inoculated for each dilution. "C.T.D.J." stands for "1/10 Celite-treated diseased juice." See text for details of preparation.*

Preparation	Dilution for inoculation	Percentage diseased
Control—diseased juice	1/1000	40
C.T.D.J.	1/1000	70
C.T.D.J. plus 2.5% Nuchar 2	1/350	100
" " " " " "	1/3500	70
C.T.D.J. plus 2.5% Nuchar W	1/350	20
" " " " " "	1/3500	30
C.T.D.J. plus 2.5% Nuchar OO	1/350	100
" " " " " "	1/3500	80
C.T.D.J. plus 0.5% Nuchar W	1/350	90
" " " " " "	1/3500	20

diseased juice. It is seen that Nuchars 2 and OO at 2.5 per cent concentration have little or no inactivating effect on the virus. Nuchar W at 2.5 per cent seems to adsorb much of the virus, while 0.5 per cent Nuchar W possibly adsorbs a little of the virus. Considering the almost perfect clearing that Nuchar OO affords and its neutral reaction toward the virus, it seems that this charcoal might be of value in studies of the purification and concentration of the virus.

The inoculations noted in table 3, together with the work described under "Salting out," also indicate that either of these treatments may be followed by evaporation with no apparent deleterious effect on the virus and with enhanced clarification of the final product.

A METHOD FOR THE CONCENTRATION AND PARTIAL PURIFICATION OF VIRUS

It having been determined by separate experiments (a) that Celite partially clears diseased juice, leaving the virus in suspension; (b) that 2.5 per cent Nuchar OO in addition brings about a practically perfect clarification of the juice and still leaves the virus in suspension; and (c) that ammonium sulfate removes virus apparently 100 per cent from suspension, after which the virus can be put back into suspension by dialysis, the scheme outlined below was devised to concentrate and purify at least partially the virus of the typical mosaic of tobacco.

(1) Grind in a food chopper plants having symptoms of tobacco mosaic. Express the juice from the ground material. Dilute one volume of such juice with four volumes of water.

(2) Treat at the rate of 100 cc. of the diluted material of (1) with 10 grams of Supercel grade of Celite for 30 minutes. Centrifuge. Discard the residue.

(3) Treat at the rate of 100 cc. of the supernatant liquid of (2) with 2.5 grams of Nuchar OO. Filter through hard filter paper. Discard the residue.

(4) Treat at the rate of 100 cc. of the practically clear and colorless filtrate from (3) with 45 to 50 grams of ammonium sulfate overnight in the icebox. Centrifuge. Filter through hard filter paper. Discard the clear filtrate.

(5) Resuspend the residue of (4) in a little water to make a thin paste and then dialyze through collodion against running distilled water until the sulfates are removed.

(6) Concentrate the dialyzed material of (5) by evaporation in a current of air at room temperature. Resuspend this dry or syrupy material in a little water and centrifuge. Discard the residue. The supernatant liquid will be slightly clouded like a weak suspension of egg albumen. The supernatant liquid is the concentrated and partially purified virus of the typical mosaic of tobacco.

In the above-outlined procedure, the dilution of the juice from the diseased plants is made because the Celite at the concentration used is more efficient when the juice is diluted 1/5 or 1/10, the latter dilution being the better. However, in this work the higher dilution would be unprofitable in the steps to follow. Although 2.5 per cent Nuchar OO has a strong clearing effect with no loss of virus, the products of the Celite and Nuchar treatments are not always the same, and this seems to depend on the conditions under which the plants had grown. If the plants had grown rapidly in soil high in nitrogen, and after treating the juice of such plants with Celite and Nuchar, the product was apparently clear and colorless. Healthy plants grown under the same conditions have juice easier to clear than that from diseased plants because the former grow more rapidly and also have a smaller proportion of brown pigments than the latter. Plants, both healthy and diseased, grown

under field conditions (Southern California, where the soil is low in nitrogen) were heavily charged with these brown pigments and did not yield as nicely to these preliminary clarifications.

The ammonium sulfate forms a flocculent precipitate which is more easily filtered out by first congregating it by centrifugation. Very little if any of the virus remains in the filtrate. Any convenient amount of water may be used to take up the precipitate.

During dialysis a further precipitate is formed, part of which can be removed by decantation from the collodion sac after dialysis is completed. This precipitate is discarded. The remainder of the material is then placed in shallow vessels and concentrated by evaporation at room temperatures. Evaporation may be continued until the desired concentration is reached or until dryness. The concentrated material may then be taken up in any desired amount of water and the suspension is then centrifuged and the residue discarded. The remaining supernatant liquid contains the virus. If the plants have not grown "normally," this final product will be colored and more or less opaque. It is possible that if 1/10 Celite-treated diseased juice were used when the original juice is highly charged with brown pigments, much of the coloring in the final product might be eliminated.

Reference to table 4 will show the results of a few typical experiments following the procedure just discussed. These data illustrate several interesting

TABLE 4. *Several experiments showing the effects of concentration and partial purification on the virulence of the virus of tobacco mosaic. Ten plants were inoculated for each dilution. "C.D. residue" stands for "concentrated and dialyzed residue from ammonium sulfate treatment."*

Preparation	Dilution of concen- trate for inoculation	BY concentrated from 6850 cc. to 52 cc.		AG concentrated from 140 cc. to 6 cc.		AU concentrated from 325 cc. to 8 cc.	
		Dilution on orig- inal basis	% dis- eased	Dilution on orig- inal basis	% dis- eased	Dilution on orig- inal basis	% dis- eased
Control		1/1000	70	1/1000	90	1/1000	40
"		1/10000	30			1/10000	0
C.D. residue	1/10000	1/76	50	1/429	50	1/246	40
"	1/25000	1/190	40			1/216	20
"	1/50000	1/380	40	1/2145	10	1/1232	40
"	1/100000	1/760	60	1/4290	20	1/2464	0
"	1/250000	1/1900	20			1/6160	0
"	1/500000	1/3800 *	25	1/21450	0	1/12320	0
"	1/1000000	1/7600 †	0				

* 20 plants inoculated.

† 30 plants inoculated.

points in relation to these studies and some of the factors that must be considered in computing the amount of concentration that has occurred.

First, it is shown that the concentration (in volume) has an effect on the amount of virulence that may be expected from the final product. This is

illustrated in the amounts of concentration in proportion to virulences at the higher dilutions of the concentrates in experiments BY and AG, although the virulences of the original diseased juices are of the same order of magnitude.

Second, it is seen that the virulence of the concentrated product is affected by the virulence of the juice from which the concentrate is made. Compare AU with AG, where the final virulences are about equal; but the concentration (in volume) of AU is about twice as great as that of AG, while the original virulence of the former is about one-half of the latter.

Third, in some cases the virulence of the virus in the concentrates approaches the same virulence as that of the original diseased juice; but on the whole the indications are that some of the virus is lost during the concentration. This result is different from that reported by most other observers. They report that their purified preparations of virus are apparently as virulent when made up to the volume of the material with which they started as was the original material. But in this study the concentrate was always made much more dilute for inoculation than 1/1 diseased juice. In the present study 1/1 diseased juice inoculated as such would undoubtedly give 100 per cent infections, while if the final concentrated preparations were restored to their original volumes, they would in some (but not all) cases give 100 per cent infections when inoculated into the plants. In order to determine by this type of inoculation the actual relative virulences of the original diseased juice and the concentrates, it seems that when inoculating with them a small amount of inoculum should be used and at dilutions which are at the threshold or beyond—i.e., do not usually yield 100 per cent infections. In this way "saturated suspensions," which would give 100 per cent infections anyway, are not compared. A comparison of relative virulences can be had by referring to paired columns under each experiment in table 4. Concentrated virulence as great as or greater than that reported under BY (table 4) has been achieved in these studies. AU and AG are both quite pure and about equally so, while BY cannot be considered as pure as the first two preparations.

TESTS FOR PROTEINS

There seems to have been no definite protein reaction reported by other workers in their purified viruses, although Vinson and Petre (1931) find a likely correlation between the amount of nitrogen in their purified products and that of some of the simple proteins.² The failure to report proteins in purified preparations of virus might be due to either one of two causes: (a) that the viruses in the preparations were so dilute that the methods used could not detect the virus, or (b) that the virus is not proteinaceous in character.

To a number of preparations of virus of different concentrations, and at least partially free of extraneous materials, were applied several of the com-

² See also Vinson, C. G., and E. J. Gildehaus. *Phytopathol.* 22: 29. 1932.

mon tests for proteins in a qualitative manner. Nessler's reagent and the biuret test were not found satisfactory. The Almén (Halma and Haas, 1929), ninhydrin, and xanthoprotein tests were used successfully and in most cases gave confirming reactions for any one preparation. Almén's reagent was found especially useful. With it the preparations from diseased plants formed precipitates with varying shades of red. After a few observations it often was possible to estimate rather accurately whether or not a sample had shown a high biological test for virus. This was done by comparing the amounts of the precipitates and the intensity of their colors.

In table 5 are given the results of the tests on a few of the preparations. In general, the concentrates made from healthy plants gave negative reactions with the reagents used. In the case of BX, the plants were of the same age

TABLE 5. *The comparative reactions for proteins in concentrated preparations of juice from both healthy and diseased tobacco plants as indicated by three different reagents. ++ = a very strong positive reaction, + = a definite positive reaction, tr = trace of protein, — = no reaction.*

Experi- ment	Kind of material	Tests			Biological reaction of the concentrate
		Xanthoprotein	Almén	Ninhydrin	
BY	Diseased	+	++	++	High virulence
AG	"	tr	+	tr	Virulent
AU	"	++	++	+	"
V	"	tr	tr	+	Low virulence
BX	Healthy	tr	+	tr	Negative
BS	"	—	—	—	"
AI	"	—	—	—	"
BZ 1/1	Diseased	++	++	++	High virulence
BZ 1/10	"	+	+	tr	
BZ 1/1000	"	—	tr	—	

and grown under the same conditions as those of BY. A comparison of the protein reactions of these two shows a difference in the amount of proteins present. When BX is compared with AI and BS (all prepared from healthy plants), an apparent discrepancy in the validity of the tests will be noted. In two cases the plants were grown rapidly in the greenhouse, while those of BX were grown in the field and were very heavily charged with brown pigments which were not completely removed in the preliminary clarifications. This again points to the importance of the conditions under which plants are grown when using them for comparative tests or in making comparable purified preparations.

Referring further to the table, it is seen that in successive dilutions of BZ the protein present is proportionately difficult to detect. A dilution of 1/1000 in this case is equivalent to a dilution of less than 1/3 on the basis of the volume of original juice used. Furthermore, this preparation was not deemed as pure as certain of the other preparations listed in the table. This would lead to the conclusion that if virus is to be detected by ordinary protein reactions, the virus must be concentrated. An example of a virus concentrate

giving both low protein and biological tests is seen in experiment V as compared with those in experiment AU. In addition, the preparation in V was not prepared according to the final method for concentration and purification (as was AU) and was not considered as pure as AU.

A word might be in place concerning the purity of these preparations. When considered on the basis of dry and ash weights per unit volume of concentrate, they are comparatively pure, but can in no way be considered chemically pure. A few dry and ash weights have been determined indicating this fact. However, if the concentrates were diluted to the original volumes of diseased juice, their ash and dry weights would compare very favorably with those found by other investigators for their preparations.

The value of the present protein tests for the virus probably is to be found mostly in indicating that purified virus preparations will have to be concentrated before any protein in the constitution of the virus can be detected. It is very likely in the work described here that the protein tests detected products associated with the virus rather than the virus itself.

SUMMARY

1. Direct current of 110 volts induces the rapid precipitation of part of the materials from the juice of plants having tobacco mosaic, and at the same time leaves the virus of tobacco mosaic in suspension.

2. A number of salts can be used for the partial salting out of virus similarly to the manner in which proteins are salted out. Ammonium sulfate was found to have the least inactivating effect on the virus and at the same time most completely precipitates it from suspension.

3. Certain activated carbons, when not used in too great concentration, can be used to clarify the juice of plants with tobacco mosaic, at the same time leaving the virus in suspension.

4. A method is described by which highly concentrated suspensions of the virus of tobacco mosaic may be prepared. The virus in these suspensions is at least partially purified.

5. There is a definite difference in the protein reactions of the concentrated juice from healthy plants and the juice from plants with tobacco mosaic. At present it seems more probable that the positive protein reactions of the juice from plants with tobacco mosaic are due more to the products associated with the virus of tobacco mosaic than to the virus itself.

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THE GENETICS OF *NEUROSPORA*. IV. THE INHERITANCE OF *TAN* VERSUS *NORMAL*

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Tan is a Mendelian factor in *Neurospora crassa* which produces a tan-colored substrate and reduced aerial mycelium. The *tan* character first appeared in half of the ascospores from ascus 114 (the first ascus shown in figure 1). This ascus was produced in the third inbred generation from a wild culture. All the other asci described in these three generations produced

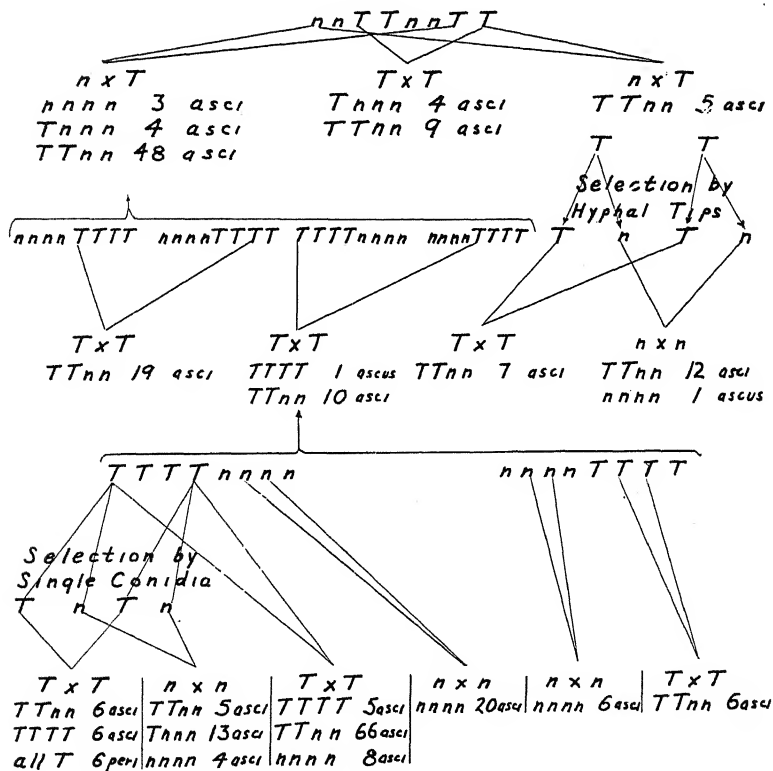


Fig. 1. Pedigree of the asci discussed in the text. Eight letters are used to designate the eight ascospores in a single ascus when the respective positions of each ascospore are shown. When a group of asci is designated, only four letters are used. Each letter stands for a pair of ascospores. In this case only the ratio, and not the position, is indicated. A bracket encloses particular individual asci of a single group.

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normal mycelia. The *tan* gene is unstable and mutates to *normal* with high frequency (Lindegren, 1933). A mating was made between a *normal* and a *tan* mycelium from this ascus. Fifty-five asci were analyzed from this mating. In 13 different cases more than one ascus was taken from a single perithecium. These asci are described in table 1. Only four letters are used to indicate each ascus because only four genotypes are found in each ascus. Ascospore 1 is always indistinguishable from ascospore 2; ascospore 3 is always indistinguishable from 4; ascospore 5 is always indistinguishable from 6; and ascospore 7 is always indistinguishable from 8. This proves that in *N. crassa* the third division is a simple mitosis and is not involved in the meiosis which constitutes the first and second divisions. A small *n* is used to designate *normal* or wild type and a large *T* to indicate *tan*. The letter *n* rather than *t* was used to designate *normal* because it seemed that some of the phenotypic *normals* were probably modified genotypic *tans*. The action of modifiers on *tan* is shown in plate 1. *Tan* mycelia 3 and 4 in ascus 278 produced dark tan substrates and much reduced aerial hyphae, while *tan* mycelia 5 and 6 produced light tan substrates and only partly reduced aerial hyphae. The action of modifiers of *normal* is also shown in plate 1. The two pairs of *normal* mycelia in ascus 278 produced very light-colored substrates, while the two pairs of *normal* mycelia from ascus 237 produced very dark-colored substrates. Table 1 shows that asci 237 and 278 came from different perithecia. The seven asci from perithecium 233 resembled ascus 237 in the darkness of the *normal* substrates, and the three asci from perithecium 277 resembled ascus 278 in the lightness of the *normal* substrates.

Some asci do not contain an equal number of *tan* and *normal* ascospores. Ascus 243 resembles ascus 278 except that 5 and 6 are *normal* instead of *tan* modified toward *normal*. It is possible that 5 and 6 (or either 1 and 2 or 7 and 8) are genotypically *tan* plus a modifier. In ascus 243 this modifier may be segregated from its allelomorph in such a way that only one of the two genotypic *tan* pairs is affected. An ascus such as 244 might be produced if these modifiers were segregated so that both of the genotypic *tans* were modified to *normal*.

Another cross between *normal* and *tan* was made by mating the mycelia from the first and eighth ascospores in ascus 114. Five asci were dissected, and each ascus contained four *tan* and four *normal* ascospores. A *tan* by *tan* cross was made by mating the third and eighth ascospores in ascus 114. Thirteen asci were dissected. Nine contained four *tan* and four *normal* ascospores. Four contained two *tan* and six *normal* ascospores. The fact that the *tan* by *tan* mating produced a large number of *normal* progeny is probably due to the mutation of the *tan* genes to *normal* in some of the nuclei as well as modification of genetically *tan* mycelia to *normal* by modifying genes.

Two *tan* by *tan* matings produced the next generation. These *tan* mycelia were selected from the group of 55 asci. In the first case 19 asci, each of which contained four *tan* and four *normal* ascospores, were produced.

TABLE I. *Character of mycelia produced by ascospores from fifty-five asci of the second generation*

Perithe- cium	Ascus	Ascospores				Perithe- cium	Ascus	Ascospores			
		1, 2	3, 4	5, 6	7, 8			1, 2	3, 4	5, 6	7, 8
218	217	T	T	n	n	258	254	n	n	T	n
	218	T	T	n	n		255	n	T	n	T
	219	T	T	n	n		257	T	n	T	n
	220	T	n	T	n		258	n	T	T	n
	221	n	T	T	n		259	n	T	T	n
	222	n	T	n	T	260	263	n	T	T	n
	223	T	T	n	n		264	n	T	T	n
	225	T	n	n	T		265	n	T	T	n
228	226	n	T	n	T		266	n	n	T	T
	224	n	T	T	n		267	T	n	T	n
	228	n	T	n	n		268	n	T	T	n
230	229	n	n	n	n	275	271	T	T	n	n
	230	n	n	T	T		274	T	n	n	T
	231	n	n	T	T		275	n	n	T	T
233	232	n	T	n	T	277	276	n	n	T	T
	233	T	n	n	n		277	n	T	n	T
	234	n	n	n	n		278	n	T	T	n
	235	n	T	T	n	281	280	n	T	n	T
	236	T	n	n	T		281	T	T	n	n
	237	n	n	T	T		282	T	n	n	T
	238	n	n	T	T	284	284	n	n	T	T
	239	T	n	n	T		285	T	n	n	T
243	240	n	T	n	T		286	n	n	T	T
	242	T	n	n	T	291	289	T	n	T	n
	243	n	T	n	n		291	n	n	T	T
	244	n	n	n	n		292	n	T	T	n
251	249	n	T	T	n						
	252	n	T	n	T						
	253	T	T	n	n						

In the second case 11 asci were dissected. Ten contained four *tan* and four *normal* ascospores. One contained eight *tan* ascospores. The fact that these *tan* by *tan* matings both produced so many *normal* progeny can be explained if we assume the parents to be heterokaryotic due to the mutation of the *tan* gene in some of the nuclei to *normal*. This explanation is supported by the fact that the first polysporous transfer from a *tan* mycelium (after growing from an ascospore) always produces a culture in which the *tan* character is either less marked or in which a complete reversion to *normal* occurs.

If the *tan* mycelia were heterokaryotic, it should be possible to separate the *tan* from the *normal* nuclei by somatic segregation. This idea prompted the next experiment. In the second generation (fig. 1) there is a group of five asci resulting from a *normal* by *tan* mating. One of these asci produced *tan* mycelia of opposite sex. Two such *tan* mycelia were selected and the conidia were sown on separate Petri dishes. Subcultures were made by cutting off hyphal tips growing out from these masses of conidia. These subcultures varied from well-defined *tan* through intermediate to well-defined *normal*. One of the well-defined *tan* mycelia and one of the well-defined *normal*

mycelia were selected as parents for further subcultures. Conidia from these extreme types were again sown on agar in separate plates and hyphal tips removed. This was done in the case of each of the originally selected *tan* lines, so that four kinds of cultures were obtained: (1) *tan* from a (+) *tan* culture, (2) *normal* from a (+) *tan* culture, (3) *tan* from a (—) *tan* culture, (4) *normal* from a (—) *tan* culture.

One of the *normal* lines produced no more *tan* mycelia after four clonal "generations." Both *tan* lines produced some *normal* subcultures even after seven clonal "generations." Over 400 subcultures were made. A *tan* by *tan* cross between two of the selected *tan* mycelia from the last clonal "generations" was made. Seven asci were dissected. Each contained four *tan* and four *normal* ascospores. A *normal* by *normal* mating was made between the mycelia from two of the selected *normal* lines. Thirteen asci were dissected. Twelve contained four *tan* and four *normal* ascospores. One contained eight *normal* ascospores. This experiment indicated that, if heterokaryosis were the cause of this type of genetic behavior, hyphal tip selection was not efficient in separating the several kinds of nuclei. When *Neurospora* conidia are sown together, the young hyphae anastomose with each other, so that the nuclear population in the hyphal tips produced from such a mycelium would be heterogeneous. It was thought that somatic segregation of the two types of nuclei could be effected more readily by single conidium cultures. However, since each conidium contains a large number of nuclei, it was not expected to get stable strains in the first clonal "generation."

With this in view, the *tan* mycelia from the first and fourth ascospores of one of the third-generation asci (fig. 1) were chosen. Subcultures from single conidia were selected for extreme *tan* and extreme *normal* for several clonal "generations." In contrast to the preceding experiment, much more stable strains were obtained. A *tan* by *tan* mating between two of the selected *tan* strains was made. Twelve asci from five perithecia were dissected. Random ascospore cultures were made from six separate perithecia, making a total of 11 perithecia in all. Six of the asci (from two perithecia) produced eight *tan* ascospores. Six other asci (from three perithecia) produced four *tan* and four *normal* ascospores. The random ascospores from the six perithecia (10 to 15 were grown from each perithecium) produced only *tan* mycelia. These data show that only two types of perithecia were found: (1) three perithecia containing *TTnn* asci and (2) eight perithecia containing *TTTT* asci. No asci were found containing eight *normal* spores. It is clear that selection of the parents by single conidium cultures increased the number of *tan* progeny.

A *normal* by *normal* mating was made between two of the *normal* mycelia which had been selected by somatic segregation from the supposedly heterokaryotic *tan* clones. Twenty-two asci were dissected. Four contained eight *normal* ascospores. Five contained four *normal* and four *tan* ascospores. Thirteen contained two *tan* and six *normal* ascospores. All but two of these

asci were from separate perithecia. One of these two was of the *TTnn* type and the other was of the *Tnnn* type. No asci were found containing all *tan* ascospores. These data show that selection toward *normal* was capable of increasing the number of *normal* progeny.

The sexual progeny of the two selected *tan* clones contained some all-*tan* asci, but no all-*normal* asci. The sexual progeny of the two selected *normal* mycelia contained some all-*normal* asci but no all-*tan* asci. Somatic segregation of *normal* from *tan* nuclei and vice versa had been effected by selection of single conidia. These conidia had been produced from single haploid ascospores. This shows that the *tan* mycelium produced by a single haploid ascospore was heterokaryotic. This heterokaryosis in a mycelium originating from haploid nuclei could only be the result of mutation of the *tan* gene to *normal*. This mutated gene was then transmitted to the sexual progeny in Mendelian manner.

Two explanations for the asci containing three-to-one ratios (*TTTn* and *Tnnn*) may be offered. One possibility is the mutation of a *tan* gene to *normal* during meiosis. Another possibility is the segregation of a gene modifying *tan* to *normal*. The fact that the two ascospores of each of the four pairs are always identical indicates that a mutation has never been detected which occurred during the third division. This may mean that mutations of *tan* to *normal* occur with a low frequency in comparison to the frequency of nuclear division, so that the chance of detecting a mutation during any specified nuclear division is not great. This indicates that the three-to-one ratios in question are probably due to modifiers. If a zygote nucleus is heterozygous for *tan* and *normal*, as well as for a pair of modifiers, the segregation of these genes at meiosis may produce three kinds of asci. The *TTnn* type of ascus is produced if the genes modifying *tan* to *normal* pass into the *normal* nuclei. The *nnnn* type of ascus is produced if the genes modifying

TABLE 2. The number of asci dissected from different perithecia produced by a mating of unselected *tan* clones

Type of perithecium	No. of asci	Type of perithecium	No. of asci
<i>TTnn</i>	11	<i>nnnn</i>	6
<i>TTnn</i>	6	<i>TTTT</i>	2
<i>TTnn</i>	5	<i>TTTT</i>	2
<i>TTnn</i>	5	<i>nnnn</i> }	{ 3
<i>TTnn</i>	5	<i>TTnn</i> }	{ 2
<i>TTnn</i>	5	<i>TTTT</i> }	{ 1
<i>TTnn</i>	5	<i>TTnn</i> }	{ 1
<i>TTnn</i>	4		
<i>TTnn</i>	4		
<i>TTnn</i>	4		
<i>TTnn</i>	3		
<i>TTnn</i>	2		
<i>TTnn</i>	2		
<i>TTnn</i>	1		

tan to *normal* pass into the *tan* nuclei. The *Tnnn* type of ascus is produced if one of the *tan* nuclei gets a modifying gene (thereby changing it to *n*) while the other does not. The *pale* gene (Lindegren, 1933) suppresses the *tan* character. When an ascus is genotypically *PT Pt pt pT*, the *tan* character is shown only by the *pT* pair of ascospores. Such an ascus produces two *pale*, one *normal*, and one *tan* pair of ascospores. A large number of asci of this type have been found. The ascus from which the *pale* stock was developed (Lindegren, 1933) was one of these. The *TTTn* type of ascus could be produced from a homozygous *tan* zygote nucleus if two factors were necessary to modify *tan* to *normal*.

A mating between the same two unselected *tan* clones served as a check on these two experiments. Seventy-nine asci were analyzed. These asci were from 19 separate perithecia. Table 2 shows that only *TTnn* asci were obtained from 14 perithecia. One perithecium contained six all-*normal* asci. Two perithecia each contained two all-*tan* asci. Only two perithecia contained more than one kind of ascus. One of these contained all-*normal* asci together with asci which were half *tan* and half *normal*. The other contained an all-*tan* ascus together with an ascus which was half *tan* and half *normal*. No perithecia were found containing both all-*tan* and all-*normal* asci. These facts show that the nature of the ascus zygote nucleus is determined before the ascogenous hyphae are formed. Minor differences may distinguish asci from the same perithecium as a result of differences in the ascus at meiosis. Therefore, different kinds of perithecia arise by the association of genetically different nuclei from two heterokaryotic thalli before the initiation of the perithecium.

It has been shown by genetic data that the ascus nucleus in *N. crassa* is diploid (Lindegren, 1933). If the ascus nucleus is diploid, it follows that each of the two nuclei fusing in the young ascus is haploid. It seems highly improbable that the two nuclei associated at the initiation of the perithecium have fused and undergone a reduction prior to the formation of the ascus (Lindegren, 1933). If this had occurred, it is easy to show that reassortment of the genes in the meiosis previous to the formation of the ascus would operate to prevent such uniformity among the asci from one perithecium as has been described. On this basis it is indicated that the association of two nuclei at the initiation of the perithecium occurs without a fusion. The genetic data in the present paper can then be interpreted in the following manner: The *tan* gene mutates rapidly to *normal* (wild type). A haploid ascospore which at its origin contains a single *tan* gene eventually produces a mycelium with two kinds of nuclei. One kind of nucleus contains the *tan* gene. The other kind of nucleus contains the *normal* allelomorph of *tan* at the *tan* locus. When two such heterokaryotic mycelia of opposite sex are mated, two nuclei (one from each mycelium) are associated at the initiation of each perithecium. This association may be between two *tan* nuclei, two *normal* nuclei, or between one *tan* and one *normal* nucleus. This associated

pair of nuclei divides either conjugately or independently until the formation of the ascus. In each young ascus the fusion of two of the progeny of the nuclei which associated at the initiation of the perithecium occurs, producing a diploid nucleus.

The reversion of *tan* cultures to *normal* at the first transplanting is due to the fact that the *tan* gene in many of the nuclei has mutated to *normal*, and at the first transplanting these *normal* genes produce their effect. As a result the mycelium resembles *normal*. Selection of conidia can produce the typical *tan* mycelia from such a reverted culture if the selected conidia contain all, or mostly all, *tan* nuclei. Such a culture does not remain *tan* but changes again by continued mutation to a heterokaryotic mycelium. The mutation of *normal* to *tan*, however, occurs very infrequently. Selected *normal* strains are much more stable than the selected *tan* strains. *Normal* mycelia, which have been derived from *tan*, when inbred become completely stabilized. Two *normal* by *normal* matings (fig. 1) were made between third-generation mycelia. The second-generation parents had been both *tan*. One mating produced 20 all-*normal* asci, the other produced six all-*normal* asci.

Table 2 shows that three main classes of perithecia were produced: (1) those containing *TTnn* asci; (2) those containing *TTTT* asci; and (3) those containing *nnnn* asci. Two mixed perithecia were found. One contained an ascus of the *TTTT* type together with an ascus of the *TTnn* type. The other contained asci of the *nnnn* type together with asci of the *TTnn* type. The first perithecium apparently contained ascus zygote nuclei homozygous for *tan* and heterozygous for a modifier of *tan*. The second perithecium probably contained ascus zygote nuclei heterozygous for *tan* and a modifier of *tan*.

The *TTnn* perithecia in table 1 were further divisible into three main classes: (1) perithecia containing four extremely light *tan* and four *normal* ascospores in each ascus; (2) perithecia containing four light *tan* and four *normal* ascospores in each ascus; and (3) perithecia containing four *tan* and four *normal* ascospores in each ascus. The classification of these different types of *tan* was very sharp. If these three main types of *tan* were the result of modification by closely linked, stable genes, modifying *tan*, one would not expect to find these modifiers distributed to the various perithecia in the manner described. These various types of *tan* seem to indicate that the *tan* gene itself is different in these different types of perithecia. This may indicate that the original dark *tan* gene has mutated to several different kinds of *tan* genes—i.e., a series of multiple allelomorphs.

Although the genetic data presented here indicate that in *N. crassa* two haploid nuclei associate at or before the initiation of the perithecium without fusing and divide conjugately until they finally fuse in the young ascus, the writer does not consider such findings as incompatible with or contradictory to the cytological findings of Gwynne-Vaughan (1932) and Harper (1905) concerning the existence of a fusion at the initiation of the perithecium.

It is possible that there are many different ways in which perithecia and asci are initiated; and the cytological findings of Gwynne-Vaughan as to a brachymeiosis in the third division, if corroborated, may throw real light on the function of the third division in the ascus of *Ascobolus*, although it is clear from the writer's genetic study of *N. crassa* that this division in *N. crassa* is merely an ordinary equational division. It is unsafe to generalize on the problem of the nuclear and sexual mechanism of the Ascomycetes from a study of one species, as Dodge (1927) has shown by his comparative cytological study of *N. crassa*, *N. sitophila*, and *N. tetrasperma*. Unless it is established that two workers were studying genetically identical cultures, it is hazardous to assume that cytological study should reveal identical nuclear mechanisms. Simply comparing collected species with the accepted taxonomic criteria may be misleading.

Gwynne-Vaughan (1933) studied the nuclear divisions in the ascus of *Lachnea scutellata*. Final analysis of this peculiar meiosis depends on knowing how many strands make up each chromosome of the definitive nucleus. Cytological study is usually not able to solve this question. In a standard meiosis there are four strands in each such chromosome, and the writer has shown by genetic analysis that the first-division metaphase chromosomes in *N. crassa* are four-parted, as in a standard meiosis. If it is possible to obtain genetic data in the case of *Lachnea scutellata*, they should be very interesting.

SUMMARY

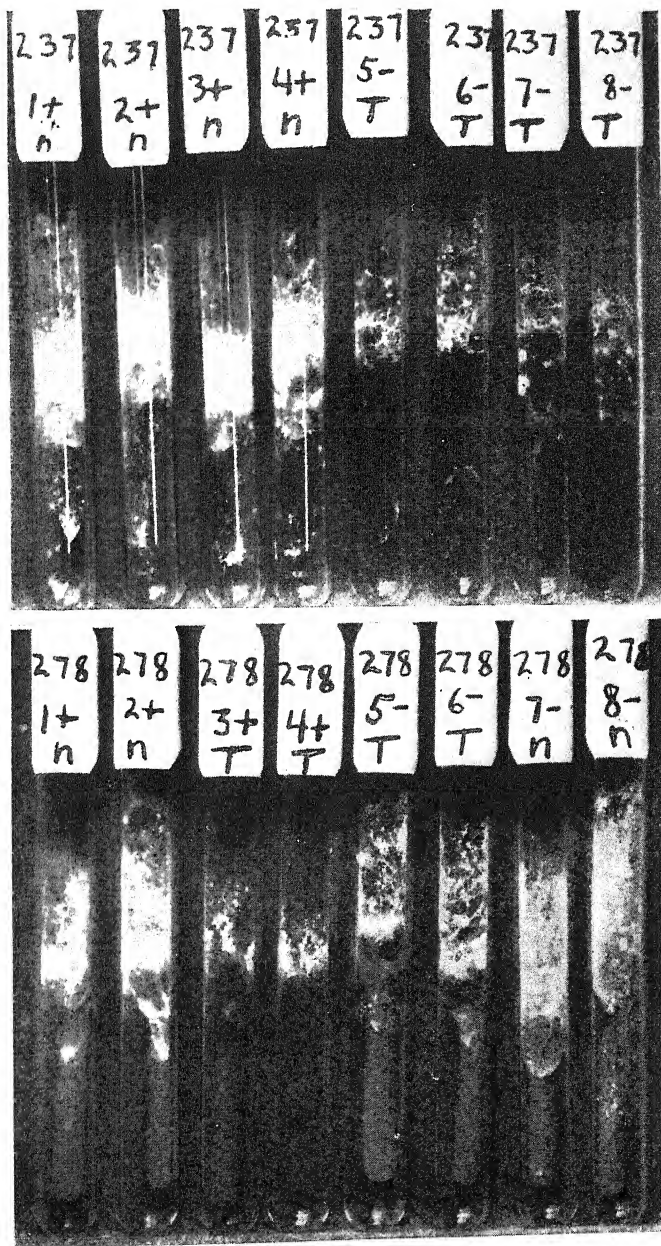
Tan is a gene in *Neurospora crassa* which mutates with high frequency to *normal*. A haploid *tan* ascospore produces a heterokaryotic mycelium containing both *tan* and *normal* nuclei. By mating two such mycelia it was possible to show that two nuclei, one from each mycelium, are associated before the ascogenous hyphae are formed, and these associated nuclei divide either conjugately or independently until the formation of the young ascus. There a fusion of these nuclei occurs to form the diploid ascus zygote nucleus.

The writer is grateful to Dr. T. H. Morgan for his continued interest and support.

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EXPLANATION OF PLATE 1

Mycelia grown from the ascospores from two of the asci obtained by mating of *normal* by *tan*. The number at the top on the label is the ascus number. The second row shows the position of the ascospore in the ascus and the sex of the mycelium. The letters *T* and *n* indicate the predominant cultural characters, *tan* or *normal*. These photographs show the extremes of variation. The *tan* mycelium in 278-5 is extremely light and in 237-7 is extremely dark. The substrate of the *normal* culture is practically colorless in 278-7 and dead black in 237-3. It is obvious that modifiers affect both the *tan* character and its *normal* allelomorph. In ascus 278 *tan* and *normal* were segregated at the second division. At least one modifier was segregated differently, causing *normal* mycelia 1 and 2 to be different from *normal* mycelia 7 and 8, and *tan* mycelia 3 and 4 to be different from *tan* mycelia 5 and 6. Both members of each pair are identical. This marked similarity of the two members of each pair is true of all the rest of the 55 asci shown in table 1.

BOAT CATCHES OF MARINE PHYTOPLANKTON IN SOUTHERN CALIFORNIA IN 1928

WINFRED EMORY ALLEN

(Received for publication January 17, 1933)

INTRODUCTION

This is the seventh in a series of reports dealing with material collected by boat in the summer seasons at two particular stations and at numerous miscellaneous stations in the Southern California region, mostly in the Gulf of Santa Catalina and near La Jolla. The essential features of the methods of collecting material have remained the same as in 1921 (Allen, 1923), the most important change being in closing bottles, the lighter and simpler Allen closing bottle having been in use since June, 1926. Because of the speed and certainty of operation of the smaller bottle it became possible to take large numbers of catches at numerous depths in a short time.

However, the increasing demands of laboratory experimentation and other activities ashore in the Institution have more than offset the advantages of such facilities in the work at sea, and it seems probable that 1928 is the last year in which there will be any pretense at continuity of operation at the two particular stations five and ten miles offshore near La Jolla.

STATIONS 1 AND 2

In 1928 work at sea did not begin until July 5, when a series of catches at Station 1 (10 miles) between the depths of one hundred meters and the surface was interrupted by a broken winch. From July 23 to August 6 the ten-mile station was occupied five times and the five-mile station (Station 2) three times. In all cases the sixteen catches at different levels from one hundred meters to the surface were too small to have any quantitative significance in relation to occurrence and distribution of either diatoms or dinoflagellates. In fact, no specimens of either group were found at most levels sampled.

THE AUGUST CRUISE

The August cruise was intended to duplicate approximately the August cruise of 1926 (Allen, 1928). It was made at about the same time in the month and followed about the same course (see map, p. 204, Allen, 1928) up the coast from La Jolla, near San Diego, to Santa Monica and across to Santa Cruz and Santa Rosa Islands (see any map of California).

As in 1926, diatoms and dinoflagellates were very few in the surface only catches made between La Jolla and Santa Monica Bay, although several

different species of dinoflagellates were sparsely represented in a number of catches.

Near the northern border of Santa Monica Bay, within five miles or so of Pt. Dume, two surface catches were taken which showed nearly 50,000 diatom cells to the liter, together with a very few dinoflagellates. However, most of these diatoms were in bad condition and the significance of their presence is uncertain.

In the vicinity of Santa Cruz Islands one fairly large catch (85,000 cells per liter) of diatoms was obtained. Others were negligible except for two taken south of the middle of the island which reached a showing of 15,000 cells to the liter.

Near Santa Rosa Island, especially in Santa Cruz Channel and the area near the eastern and northern parts of the island, a number of catches of diatoms were made which exceeded 100,000 cells to the liter, but no significant numbers of dinoflagellates were found. The numbers of diatoms were mainly due to small *Chaetoceros* spp. in poor condition.

The largest catch of the cruise (and of the season for the boat) was made near Santa Barbara, where over 400,000 diatom cells to the liter were found. These also consisted mainly of small *Chaetoceros* spp. in poor condition. On the return trip to La Jolla no large catches were obtained, but a few did show more than 1,000 diatom cells to the liter. Near Catalina Island, series taken at depths between one hundred meters and the surface showed nothing that had numerical significance.

IRREGULAR SERIES

After finishing the August cruise the boat returned to her permanent mooring in San Diego Bay. She made two short trips to sea from there in October on which all catches were below 1,000 cells to the liter of diatoms and dinoflagellates combined.

SUMMARY

In the way of positive results the catches of phytoplankton by boat for 1928 seem rather unimportant except for the indication similar to that of 1926 that the vicinity of Santa Rosa Island is exceptionally productive (at least in August) and the indication similar to that of several other seasons that surface catches are negligible in size in the Gulf of Santa Catalina in August. The fact that neither *Thalassiothrix frauenfeldii* (Grun.), prominent in 1926, nor *Nitzschia seriata* Cl., prominent in 1927, was conspicuous in any catch is interesting, but the subsurface catches of 1928 were too few to permit comparison, inasmuch as one or the other may have been present at some depth and locality not tested.

Notwithstanding its deficiencies and the lack of significant material in most of its catches, the boat work of the Institution in 1928 may be regarded as highly valuable and important because it presents an excellent warning against making or accepting conclusions concerning natural conditions before

sufficient evidence has accumulated. From the considerable number of catches taken at numerous stations widely distributed, and from the several series of catches at different depths, one might feel warranted in saying that the Southern California region was non-productive of ocean pasturage, at least in 1928. In fact, some generalizations about oceanic conditions rest on foundations less representative than these. But in this case it is easy to show that the catches were all taken in one of the less productive periods of the year, and that the year as a whole was more than ordinarily productive, at least at points near the north and the south limits of the territory covered. The records of pier catches at both Pt. Hueneme (near the north) and at La Jolla (near the south) show that at both pier stations the abundance of diatoms had reached a week average of more than one million cells per liter in March and week averages of hundreds of thousands at two times between March and the period of the August cruise. They also show light abundance at both piers coinciding with the time of the cruise. In addition, it is notable that in most years the time of the cruise (in the latter part of August) falls in a period of very low abundance of marine plankton diatoms in Southern California waters at surface levels, and probably at other levels. Confronted with phenomena such as these, one is driven to the view that the August cruise yielded little information concerning possibilities of annual production in the region traversed and that generalizations concerning productivity in other parts of the year based on its evidence are valueless.

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ALKALI SCORCH OF BERMUDA ONIONS¹

J. J. TAUBENHAUS AND WALTER N. EZEKIEL

(Received for publication January 24, 1933)

Specimens of white Bermuda onions were received from a produce house at Austin, Texas, in 1928 with the following statement: "These onions are apparently sound and of good quality but a good portion of them, mainly those coming in contact with the bags, bear a brown stain resembling a burn. We are unable to make delivery of the car as the consignee is afraid the condition existing was brought about by some chemical which may have poisoned the onions or rendered them unfit for human consumption." Onions affected similarly have been received since from Texas railroads and from commission houses in San Antonio, Houston, and Dallas.

Affected onions are recognized by the irregular, superficial, brown to dark reddish spots on the outer scales of the bulbs (fig. 1). Under dry storage conditions the spots gradually work in deeper, finally involving entire bulbs, which dry and become mummified.

No organism could be isolated from the discolored outer scales nor from the affected scales underneath in the more advanced injury which developed in storage. It was therefore suspected that the trouble might be due to toxic substances in the bags used for storage and shipping. Analyses were made of a number of the jute bags in which the original affected specimens had been shipped.² A water extract from the bags gave a pH value above pH 9.6 (the limit with the colorimetric outfit used for determination). The extract contained 6.3 per cent of solid matter, and titration showed that it was as caustic as a solution of 1.8 per cent of sodium hydroxide. Other tests were made which suggested that the sacks did contain a considerable amount of caustic soda, probably partly combined with the sacking and onion materials. On the other hand, a water extract from new clean jute fertilizer sacks had a pH of 5.4 and contained only 0.1 per cent of solid matter.

Two of the jute bags from which the affected onions had been removed, and two other bags which were of the same shipment but had contained only uninjured onions, were used for an experimental test of the cause of the trouble. Normal Bermuda onions received freshly from a grower at Laredo, Texas, were placed in each bag. After five weeks' storage in the laboratory at room temperature, the sacks were opened, and typically scorched onions

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² Analyses were made through the courtesy of Dr. G. S. Fraps, Chief of the Division of Chemistry, Texas Agricultural Experiment Station.

found only in the bags which had originally caused the trouble. No scorched onions were found in the check bags. The alkaline contaminations in the sacks were therefore probably the cause of the injuries noticed.

Apparently similar types of injury have been described recently by Ramsey³ as "scorched spot" and "bag paint." He attributed these superficial

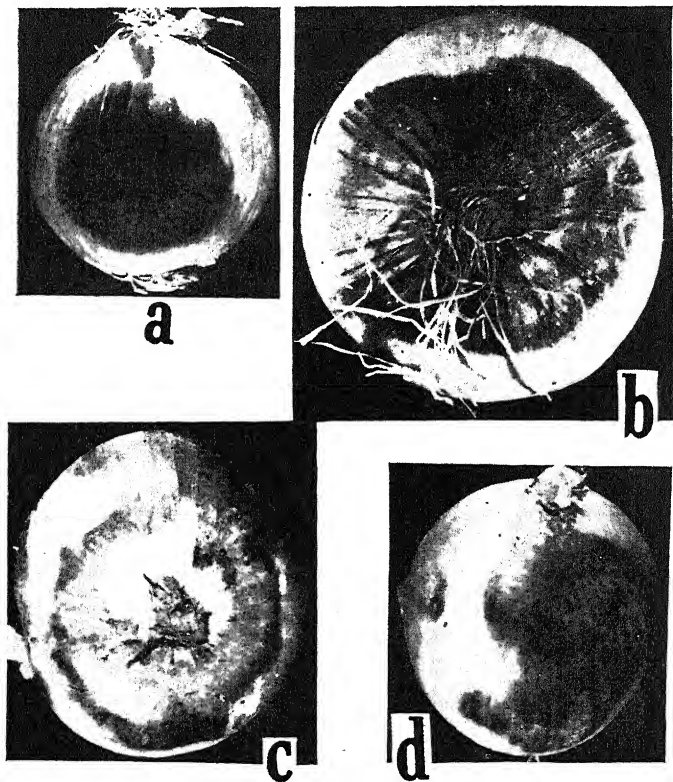


Fig. 1. Alkali scorch of white Bermuda onions: *a* and *c*, with scorched areas on the side; *b*, with scorched area on the plate of the bulb; and *d*, with outer scorched scales removed to show early stage of spread of the injury to the inside of the bulb.

discolorations to chemicals in the fabric of the bags, and stated that he had not observed injuries "to the underneath fleshy scales that would impair the eating qualities of the onions." The observations recorded in the present paper, however, have shown that, particularly when the injured onions are left in storage for several weeks, the scorch works gradually into the inner scales and eventually destroys the affected bulbs.

³ RAMSEY, G. B. Blemishes and discolorations of market onions. U. S. Dept. Agr. Circ. 135: 1-4. 1930.

SUMMARY

A shipping and storage injury of onions, named "alkali scorch," has caused appreciable loss of Texas-grown white Bermuda onions. The injury resulted apparently from heavy impregnation of alkaline materials in the jute bags used for onion storage or shipment. The trouble was reproduced experimentally by placing sound onion bulbs in such bags.

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GROWTH OF *LUPINUS ALBUS* SEEDLINGS IN SOLUTIONS OF SOME AMINO-ACIDS

DAVID I. MACHT

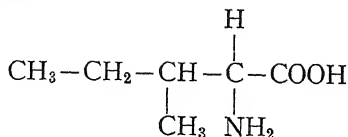
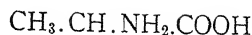
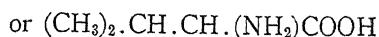
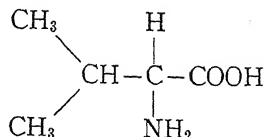
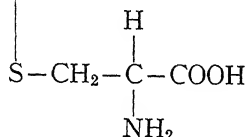
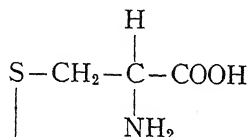
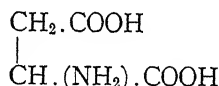
(Received for publication January 25, 1933)

INTRODUCTION

For a number of years the author has been engaged in the study of the effects of various drugs, poisons, and chemicals in general on the growth of living plants. These studies have led to some interesting discoveries. It was found that certain alkaloids which are very poisonous for animal tissues are comparatively little toxic for living plants (Macht and Livingston, 1922). On the other hand, it was found that certain products of metabolism in the blood of animals are very poisonous for living plants and yet practically non-toxic for living animal protoplasm (Macht and Lubin, 1924). The author further demonstrated that chemical isomers may produce quantitatively different effects when studied on living plant tissues (Macht, 1930), and that even stereoisomers may be differentiated from each other by studying the growth of living seedlings in solutions of such isomers (Macht, 1922). Still other studies revealed that combinations of stereoisomers, when used on either animals or plants, in certain cases produce synergistic phenomena (Macht, 1929a). These investigations suggested a study concerning the effect of a number of stereoisomeric amino-acids on plant test objects. The method was to study the growth of the single, straight, well-defined roots of the seedlings of *Lupinus albus* in plant-physiological solutions with and without an admixture of one of the chemicals. Plant physiologists have found that *Lupinus albus* is especially adapted for quantitative investigations because the seeds of this plant give practically one hundred per cent of germination, and the growth of the seedlings may be conveniently measured. A brief report on the effects of two varieties of leucine and three of cystine has been published elsewhere (Macht, 1929b). In the present study, in addition to leucine (β -methyl- α -amino-valeric acid) and cystine (β -thio- α -amino-propionic acid), the isomers of three other amino-acids were studied—namely, alanine (α -amino-propionic acid), valine (α -amino-isovaleric acid), and asparaginic acid (α -amino-succinic acid). The chemical structure of these compounds is well known and is expressed by the following formulae:

METHODS

The stereoisomers l- and dl-leucine were obtained in highly purified form from the research laboratories of the Eastman Kodak Company. Three varieties of cystine, d-, l-, and dl-cystine, were obtained through the courtesy

Leucine*Alanine**Valine**Cystine**Asparaginic Acid*

of Professor Wright Wilson of the University of Pennsylvania. D- and dl-alanine, d- and dl-valine, and l- and dl-asparaginic acid in chemically pure form were obtained from the research laboratories of Hoffmann, La Roche, Inc. These substances were dissolved in equal parts of Shive's solution and distilled water, and their effects were studied by the technique of Macht and his co-workers on living seedlings of *Lupinus albus*. Shive's medium is a solution containing all the elements necessary for the growth of plants (Shive, 1915). Its composition is fully described by the author elsewhere; and it need only be stated here that it has concentrations of calcium nitrate, magnesium sulphate, and di-acid potassium phosphate in definite proportions. When such a solution is mixed with an equal volume of distilled water, its hydrogen ion varies from pH 4.6 to pH 4.8. Leucine was found to be readily soluble in such a mixture in concentrations of 1:100, and even of 1:80. Cystine is extremely insoluble, and stronger solutions of this substance than 1:25,000 could not be made even by heating. Solutions of the two alanine specimens were studied in concentrations of 1:1000 and 1:2000; solutions of the two valines were employed in concentrations of 1:2000; and solutions of the two specimens of asparaginic acid, which is less soluble than alanine and valine, were studied in concentrations of 1:4000. Even though all the amino-acids mentioned, with the exception of leucine, were poorly soluble, they were found to produce definite effects on the growth of *Lupinus* seedlings.

The procedure employed was as follows. Great care was taken to germinate the seeds in finely ground sphagnum moss in the dark. Only perfectly healthy seedlings were used for making the tests. These were selected

with special care and "matched up"; that is, only seedlings of nearly the same initial length were employed, in order to insure the greatest accuracy and to detect even slight variations in their growth produced by the various solutions. Seedlings with roots measuring from 35 to 45 mm. were employed. Ten seedlings were measured and immersed in normal nutrient solution. Other series of ten seedlings were measured in the same way and immersed in solutions of the nutrient medium plus the different amino-acids to be tested. Having been measured and immersed in the various solutions, in hard-glass test tubes, the seedlings were placed in the dark at a constant temperature for twenty-four hours. At the end of that time the increment of growth of the roots was measured. The growth of the seedling roots in solutions of the various amino-acids was compared with that of the normal controls, and the former was expressed as percentage of the latter. Such percentages are designated as the indices or coefficients of growth for the respective amino-acids examined.

RESULTS

Table 1 exhibits the results obtained with *Lupinus albus* seedlings in various concentrations of the amino-acids studied. It may be well to state that careful controls were made to determine whether small differences in the hydrogen-ion concentrations of the Shive solutions containing the various chemicals were responsible for the differences noted in the growth of the seedlings. Many previous experiments performed by the author and his collaborators in this connection showed that changing the hydrogen-ion concentrations of normal Shive's solution produced very little difference in the growth of plants within considerable limits. The hydrogen-ion concentrations of the amino-acids studied were determined with a hydrogen electrode potentiometer. These showed only slight deviations from the hydrogen-ion concentration of normal Shive's solution, so that they could not be held responsible for either the stimulation or inhibition of growth observed in the experiments. Table 1 is a composite of a large number of experiments.

It will be noted that only leucine was rather freely soluble in the solution. The other compounds studied were employed in concentrations of 1:1000 or less. Cystine, being very insoluble, was used in a concentration of 1:25,000. It was found that some of the solutions inhibited growth of the seedlings, as indicated by the phytotoxic indices, while others had little or no effect; and still others actually stimulated the growth of *Lupinus albus*. Thus, in the case of leucine, concentrations of 1:80 produced a marked inhibition of growth. Concentrations of 1:1600 of two varieties of leucine, on the other hand, either produced no change or actually stimulated growth. These stimulating effects are not at all unusual in plant experiments. It is a matter of common knowledge to those studying the effects of drugs and poisons on plants that while certain chemicals are quite toxic for seedlings when used in large doses, there is actually a point of concentration at which the same substances may stimulate their growth.

One of the most interesting observations made in the experiments mentioned was the discovery of the uniformly greater potency of the dl- and l-varieties as compared with the dextro-rotatory varieties of the amino-acids. Unfortunately, the author was unable to secure all the stereoisomers in every case, but even the dl- or racemic variety was found to be more potent physiologically than the dextro-rotatory. These findings agree with observations made by the author on the stereoisomers quinin and quinidin, and on cinchonin and cinchonidin.

TABLE I

Substance	Concentration		Variety				Effect
	Per cent	Mol per liter	D-	L-	DL-	Combination	
Leucine	1.25	0.095	—	64%	91%	69% (l + dl)	Synergistic
Leucine	0.50	0.038	—	98%	125%	108% (l + dl)	Synergistic
Leucine	0.08	0.00475	—	101%	115%	104% (l + dl)	Synergistic
Cystine	0.004	0.00016	105%	92%	96%	92% (d + l)	Synergistic
Alanine	0.10	0.0110	58%	—	46%	58% (d + dl)	Antidynamic
Alanine	0.05	0.0055	83%	—	69%	78% (d + dl)	Antidynamic
Valine	0.05	0.0048	83%	—	73%	78% (d + dl)	Additive
Asparaginic acid	0.025	0.0019	—	80%	107%	81% (l + dl)	Synergistic

Interesting from the standpoint of physiology and pharmacology were the results obtained with combinations of the stereoisomers used. The table exhibits three different possibilities which may occur when combinations of two drugs, whether of a different chemical nature or isomeric with each other, are studied on biological test objects. Such a combination may give a simple summation—that is, a result explainable by the average mean of the readings given by the two component drugs. Again, such a combination may produce a synergistic effect—that is, a result showing potentiation of the one component by the other and yielding a figure not equivalent to the arithmetical mean of the results of the two constituents. Thirdly, a combination of two drugs may have an antagonistic effect, which is not explainable by the algebraic summation of the results of the two components but is the opposite of a potentiation, and is sometimes called an *antidynamic* effect, a term introduced by the pharmacologist S. Loewe. In the case of valine, a mixture of the dextro-rotatory and racemic varieties yields a result equivalent to a simple summation of the effects of the two components. In cases of leucine, cystine, and asparaginic acid there is a synergistic effect, the effect of the combination being heightened and greater than the simple arithmetical mean of the two component effects. Finally, in the case of alanine, an antidynamic effect is produced by the combination of the d- and dl-varieties; the mixture proves to be less potent than would be expected from the average mean of the read-

ings given by the two components. It was formerly believed that synergistic and antidynamic effects could be produced only by combinations of substances radically different in their structure. The writer has shown in other publications that this is not true and that synergistic phenomena may be obtained with mixtures of substances which are structural isomers and even with combinations of some stereoisomeric compounds. The results obtained with leucine and cystine, asparaginic acid, and alanine corroborate these findings.

SUMMARY

1. The growth of *Lupinus albus* seedlings was studied in solutions of stereoisomeric varieties of leucine, cystine, alanine, valine, and asparaginic acid.
2. Such solutions may either inhibit or stimulate the growth of plants, depending on the concentrations used.
3. The laevo- varieties of the amino-acids exhibit the greatest physiological activity, and the dextro- varieties the least, the dl- or racemic forms occupying an intermediate place in this respect.
4. Combinations of stereoisomeric valines produce a simple additive effect; combinations of stereoisomeric leucines, cystines, and asparaginic acids produce a potentiation; and combinations of the stereoisomeric alanines produce an antidynamic effect.

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CYTOLOGICAL STUDIES ON SOME GENERA OF THE IRIDACEAE

WM. H. BRITTINGHAM

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The Iris family contains about sixty genera, approximately half of which are or have been cultivated. Yet, considering the horticultural importance of this family of plants, very few of the genera easily available have been investigated cytologically—actually only eight, and only two of these, *Iris* and *Crocus*, in any way extensively.

This report deals with a study made on the root-tip chromosomes of twelve Iridaceae genera, eight of which have not previously been reported.

MATERIAL AND METHODS

The sources of my material were commercial seedhouses. Root-tips were grown in bulb fiber from mature corms and killed and fixed in Navashin fluid (formula: 10 per cent chromic acid, 3 cc.; 10 per cent acetic acid, 20 cc.; formalin, 1.66 cc.; and distilled water, 47.34 cc.). Fixation was very good. The usual cytological technique was followed in embedding the root-tips in paraffin. Sections were cut 12 microns in thickness and stained by the crystal violet-iodine method of Newton. Examination of the stained preparations was facilitated by the use of an Eastman K3 (yellow) filter.

Antholyza

Antholyza paniculata Klatt. The diploid number of chromosomes is 26. There are four long chromosomes, two of intermediate size, and 20 smaller chromosomes of about the same general size (fig. 1). This is the first report of chromosome numbers for this genus.

Babiana

Babiana stricta Ker. This species and its varieties are the most generally cultivated. Yamamoto (1931) has reported $2n=12$. My work confirms the diploid number of 12. Four of the chromosomes are very long, four are about a third as long, and four are quite short and plump. To my knowledge, this is the first time the chromosomes of *Babiana* have been figured (fig. 2, fig. 17).

Crocus

A recent paper by Mather (1932) reports chromosome counts on forty-three species and varieties of *Crocus*. Diploid numbers range from 6 to ca. 46. I investigated six species, in each case confirming the counts of Mather.

Crocus Imperati Tenore. Mather reported $2n = 26$. My material also gives this diploid count.

C. Korolkowii Maw and Regel. The diploid number is 20, as given by Mather. This species has given excellent material in which $2n = 20$ is unquestionable. A photomicrograph of a particularly clear plate is reproduced in figure 18.

C. Tomasinianus Herb. Heitz (Gaiser, 1930) reported $2n = \text{ca. } 18$. Mather gives the diploid count as 16. My preparation of this species shows 16 chromosomes.

C. vernus All. This is the common spring *Crocus* of the garden. Yamamoto (1931) found a diploid number of 16; Mather reported 18. Although the chromosomes of *vernus* are very long and difficult to count, it appears from my material that 18 is the correct number.

C. versicolor Ker. Mather gives a diploid count of 26. This number is confirmed.

C. zonatus Gay. A diploid count of 8 is given by Mather. This species gives fine cytological material and the count of 8 is unmistakable. The chromosomes of *zonatus* are shown in figures 3 and 19 and are reproduced to show the differences in size and shape between them and the chromosomes of this species as figured by Mather. Mine are quite thick and massive; his particularly thin and delicate. There are also greater differences in length between his long and short chromosomes than are found between mine. If the inaccurate naming of my material be dismissed as improbable, it would appear that Mather and I have obtained widely separated races of *C. zonatus*.

Freesia

Freesia refracta Klatt. This species is considered the original stock from which the present varieties and "horticultural species" have arisen. Taylor (Gaiser, 1930) reported a diploid number of 22 in the variety "Fisheri." I have also found 22 chromosomes in material of Rainbow Mixture (an assortment of colored varieties) and the variety "Purity" (pure white). The chromosomes of *Freesia* are practically all of the same size, somewhat asymmetrical, and with pointed ends (fig. 4).

A variety of *Freesia* known as "S. and W. Improved Purity" proved to be tetraploid, having 44 chromosomes. This is a new chromosome number to be reported in this genus (fig. 5, 21).

Gladiolus

Gladiolus Colvillei Sweet. This "species" originated in a cross between *G. tristis concolor* and *G. cardinalis* and contains the Baby Gladioli much prized for pot culture. "The Bride," the pure white variety, has a diploid number of 30. The chromosomes taper at the ends and are rather uniform in size, although there are perhaps four which are slightly larger (fig. 7). This is the first report for the above species, but previous work on *Gladiolus* con-

sists of a count made by deVilmorin and Simonet (Gaiser, 1930) on *G. primulinus* hyb. var. "La Muerthe" in which $n = 30$; and counts made on unnamed garden varieties by Kinoshita, reporting $2n = 30$, and by Wakuwa, reporting $2n = 60$ (Kihara and others, 1931).

Homeria

Homeria elegans Sweet. The chromosomes of this species are very large and of great length. The $2n$ number has been determined as 36. Size groups cannot be ascertained. Chromosome counts in species of this genus have not previously appeared (fig. 8).

Ixia

Ixia crateroides major Ker. The diploid number is 20. There are four long chromosomes of about the same size and sixteen which are short and plump. The *Ixia* variety "Morning Star" also gave a diploid count of 20 (fig. 9, 20).

Ixia spp. varieties "Afterglow," "Althea," "Conqueror," "Englishton," "Hubert," and "Mozart." These six varieties (species unknown but probably hybrid varieties of *I. maculata* and *I. columellaris*, since these are considered to be the most important horticultural stocks) are tetraploid, having 40 chromosomes (fig. 10).

The chromosomes of *Ixia* are here described for the first time.

Lapeyrousia

Lapeyrousia cruenta Benth. (Flame Freesia). The diploid number is 16, the chromosomes showing quite a variation in size and shape. There are four long chromosomes with no constrictions, two with a slight median constriction, two with a prominent terminal constriction, and eight smaller chromosomes (fig. 11). Counts in this genus have not previously appeared.

Montbretia

(see *Tritonia crocosmaeflora*)

Sparaxis

Sparaxis tricolor Ker. (Wand-flower). This species and its horticultural varieties are the usual cultivated forms. My material consisted of this species and an unnamed variety. The $2n$ number of both was 20. There are four long chromosomes of about the same size and sixteen smaller ones, varying slightly in length (fig. 12). This is the first cytological report on species in this genus.

If the number, size, and shape of the chromosomes of *Sparaxis* be compared with those of *Ixia* (fig. 9), it is seen that the numbers are identical and that the sizes and shapes are remarkably similar.

Tigridia

Tigridia pavonia Ker.-Gawl. (Tiger-flower, Shell-flower). The diploid number has been found to be 26. The chromosomes are rather large and thick and show considerable size and shape variation. There are four quite long chromosomes, two of medium size with a slight constriction on one end, two more of medium size with a pronounced median constriction, and eighteen smaller ones with slightly varying lengths (fig. 13). This is one of the genera investigated cytologically for the first time.

Tritonia (blazing star)

Tritonia crocosmaeflora Lemoine. This form, a bigeneric hybrid between *Tritonia Pottsii* and *Crocoshia aurea*, is generally known as *Montbretia* and occurs in practically all bulb catalogs under this name. The diploid number is 22. The chromosomes are quite definitely separated into three size classes, there being ten long chromosomes, two of which have a terminal constriction, four chromosomes of medium size, and eight rather short chromosomes (fig. 14).

T. crocata Ker.-Gawl. The diploid number is 20, there occurring four long chromosomes of approximately the same size and sixteen short chromosomes (fig. 15).

The chromosomes of *T. crocata*, in addition to being of the same number, exhibit the same size and shape characteristics possessed by those of *Ixia crateroides* and *Sparaxis tricolor*. An examination of figures 9, 12, and 15 will show not only this but also the same absolute measurements which are common to the chromosomes of these species of different genera. This suggests that these forms are very closely related. It is planned to attempt a series of bigeneric crosses among them.

Chromosome counts in species of *Tritonia* have not previously appeared.

Watsonia

Watsonia sp. horticultural variety. The diploid number is 16. There are six long chromosomes with no constrictions, two long chromosomes, each with a pronounced median constriction, and eight short chromosomes of about the same general size (fig. 16). *Watsonia* is a new genus to be investigated cytologically.

CHROMOSOMAL CHIMERAS IN FREESIA

One tetraploid cell was observed in root-tip material of the diploid *Freesia* variety "Purity." In the sectioning, the knife had cut into the chromosome plate, leaving 34 chromosomes in one section and the remaining 10 chromosomes in the adjacent section. These two complements of an original 44-chromosome plate are shown in figure 6, *a* and *b*. Tetraploid cells in tissue normally diploid have been found quite extensively in root-tips of other plants, particularly *Crepis* (Hollingshead, 1928; Navashin, 1930).

The more important and interesting chromosomal aberration was observed in the tetraploid *Freesia* variety "Improved Purity." Of four root-tips, three were perfectly normal in that all of the plates examined contained 44 chromosomes. The fourth root-tip, however, was not consistent in its chromosome counts. Material was plentiful, so that many plates could be studied. Forty division figures, the majority, showed 44 chromosomes. Figures 5 and 21 are of a tetraploid plate from this tip. The remaining chromosome plates studied, thirty-one, contained uniformly the diploid number of chromosomes, 22. The diploid cells occupied a definite sector of the root-tip sections. This region was in the shape of a wedge representing approximately one-third of the area of the section. One of these diploid plates is shown in figure 22. This $2n$ plate and the $4n$ plate shown in figure 21 were photographed from the same root-tip, two sections apart.

Unquestionably, "Improved Purity" is normally a tetraploid variety. My observations have failed to explain this reversion to the diploid condition. Winkler (1922) observed a reversion to diploidy in the tetraploid *Solanum nigrum* *gigas* which he had experimentally produced. Nemec (1926), after artificially inducing tetraploidy by the use of narcotics, found multipolar divisions in his material in subsequent mitoses. He states that a reduction of a tetraploid chromosome number to its original diploid condition is possible through four-polar mitoses and adds that, in his opinion, Winkler's results are to be thus explained.

The diploid sector found in the root-tip of *Freesia* presumably arose from a single diploid cell. The origin of this original $2n$ cell is unaccounted for. It may have arisen from a multipolar mitosis, as Nemec has shown to be possible, or it may have arisen by a pseudo-reduction division.

I wish to thank the Biology Department of Goucher College for its many kindnesses in providing facilities for this work; also Dr. R. E. Cleland, who has given valued help and criticism.

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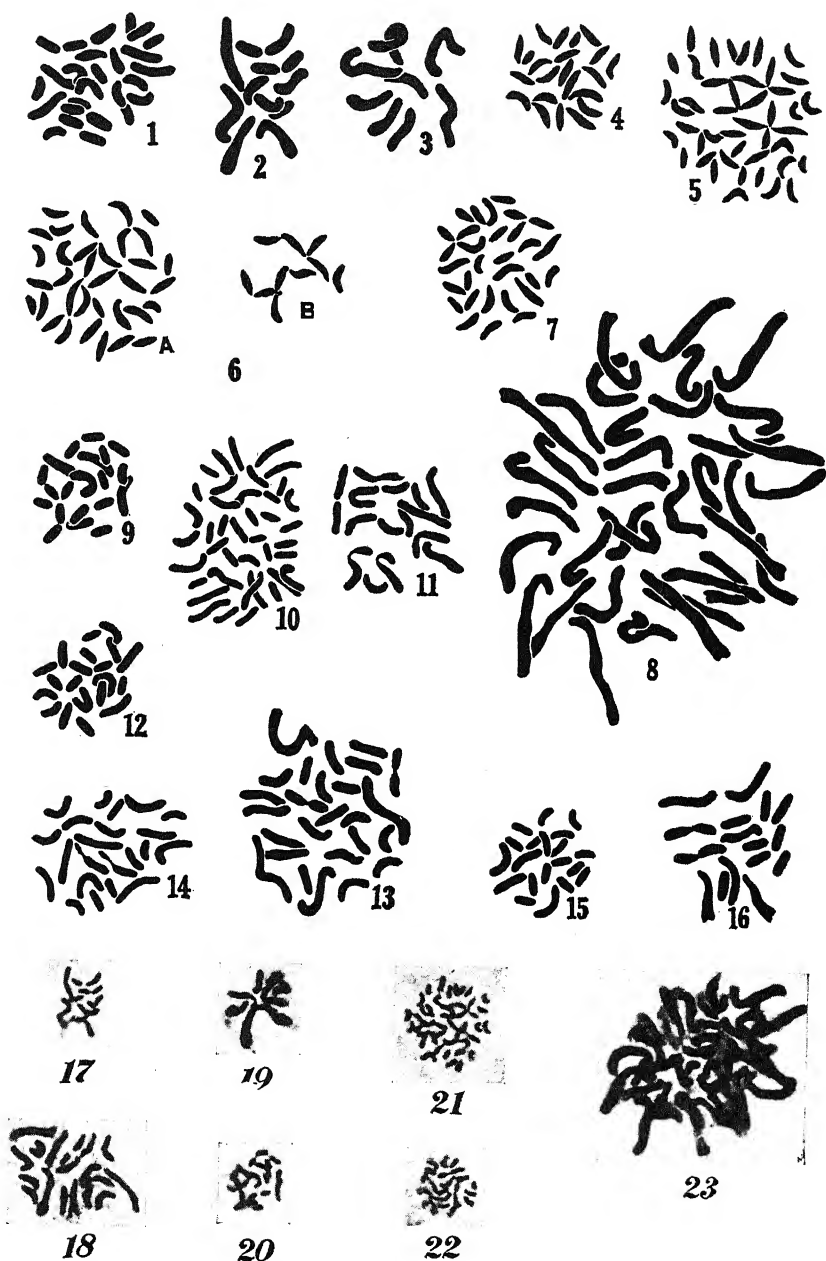
EXPLANATION OF PLATE 1

All drawings were made with a camera lucida, a Bausch and Lomb $97\times$ oil immersion objective, and $25\times$ compensating ocular. Figures are reduced one-half in reproduction to give an approximate magnification of 1640 diameters.

- Fig. 1. *Antholyza paniculata* $2n = 26$.
- Fig. 2. *Babiana stricta* $2n = 12$.
- Fig. 3. *Crocus zonatus* $2n = 8$.
- Fig. 4. *Freesia refracta*, diploid "Purity" $2n = 22$.
- Fig. 5. *Freesia refracta*, tetraploid "Improved Purity" $4n = 44$.
- Fig. 6a, 6b. *Freesia refracta*, tetraploid chimera in diploid variety "Purity."
- Fig. 7. *Gladiolus Colvillei* $2n = 30$.
- Fig. 8. *Homeria elegans* $2n = 36$.
- Fig. 9. *Ixia crateroides major*, diploid variety $2n = 20$.
- Fig. 10. *Ixia* sp., tetraploid variety "Afterglow" $4n = 40$.
- Fig. 11. *Lapeyrousia cruenta* $2n = 16$.
- Fig. 12. *Sparaxis tricolor* $2n = 20$.
- Fig. 13. *Tigridia pavonia* $2n = 26$.
- Fig. 14. *Tritonia crocosmaeflora* (*Montbretia*) $2n = 22$.
- Fig. 15. *Tritonia crocata* $2n = 20$.
- Fig. 16. *Watsonia* sp., hort. variety $2n = 16$.

The photomicrographs were taken with a Bausch and Lomb $97\times$ oil immersion objective and $15\times$ compensating ocular and are reproduced actual size of contact prints.

- Fig. 17. *Babiana stricta* $2n = 12$ (same plate as fig. 2).
- Fig. 18. *Crocus Korolkowi* $2n = 20$.
- Fig. 19. *Crocus zonatus* $2n = 8$.
- Fig. 20. *Ixia crateroides major* $2n = 20$ (same plate as fig. 9).
- Fig. 21. *Freesia refracta*, tetraploid "Improved Purity" $4n = 44$ (same plate as fig. 5).
- Fig. 22. *Freesia refracta*, diploid cell in tetraploid "Improved Purity."
- Fig. 23. *Homeria elegans* $2n = 36$ (same plate as fig. 8).



THE INFLUENCE OF VARIOUS TYPES OF DEFOLIATION AND LEAF WOUNDING UPON THE GROWTH AND YIELD OF BEANS

H. L. CHANCE

(Received for publication January 30, 1933)

The work was undertaken to determine the influence of various types of leaf wounding upon the yield of leaves, fruit, stems, and roots of beans at different stages of development. The wounding was restricted to the first pair of leaves and was done before the other leaves expanded. It consisted in the removal of definite portions of the leaf area or in the slashing of the blades. The portions were removed as a whole leaf blade, one-half or one-fourth of a leaf blade en bloc, or comparable areas in the form of disks. Two sizes of disks were removed, one having an area of one square centimeter and the other an area of one-fourth of a square centimeter.

The disks were removed with the idea of simulating insect injury, while the slashing might be comparable to hail injury. As a secondary feature, data were taken for a study of the effect of the different leaf treatments upon the time of the blooming period.

GENERAL METHODS

The beans were grown in sand contained in six-inch unglazed pots. The holes in the bottom of the pots were corked to prevent the outgrowth of roots. The sand was dried and sifted through a screen having about 20 meshes to the inch before being put into the pots.

Usually sixty pots were planted at a time. Fifty cubic centimeters of a suspension of *Rhizobium leguminosarum* were added to the sand in each pot at planting time. Ten seeds that had been wet in the suspension were planted in each pot. When the seedlings reached a stage in development where their fitness could be determined, they were thinned to eight plants to a pot. Generally no treatment, other than the addition of tap water, was given until wounding time.

From the sixty pots of seedlings, sets consisting of nine pots each were selected—one pot each for the first and second check and the seven types of wounding employed. Generally five sets were selected from the sixty pots planted. All sets selected from a single planting will constitute a group as designated in the experimental data. In selecting the members of a set, stress was placed upon uniformity within the set rather than between the sets.

The first check was harvested at the time of wounding and the second check at the time of harvesting the rest of the plants. The wounding was

done when the first pair of leaves was well developed and the central shoots were from one to two inches long.

METHOD OF HARVESTING

The leaf blades, fruit, stems, and roots were harvested separately. The central buds of the first check were weighed with the leaves, while all petioles were weighed with the stems. The fruit varied in size from pods which had just set to full-formed pods. Where the pods were harvested, the remaining flowers and flower buds were harvested with the fruit; otherwise they were harvested with the stems.

The roots were rather difficult to harvest without loss. The following method was used. After the stems were harvested, one hand with the fingers outspread was placed over the pot, which was inverted and loosened from its contents by tapping it on the work bench. The pot was removed, and a pot-shaped basket made of 14-mesh copper wire was placed over the mass of sand and roots and brought to an upright position. This was immersed in a three-gallon pail of water and agitated. As the sand was loosened, it sifted through the basket. The loosening of the sand and the buoyant action of the water upon it prevented the breaking of the roots to any great extent. After the roots were removed, they were submerged in clean water and any sand particles still adhering to them were removed by stroking with a varnish brush. The clean roots were blotted between cotton towels before being weighed. All weights are given in grams.

METHOD AND TYPES OF WOUNDING

After selections were made for a set, the leaves, stems, and roots of the first check were harvested immediately. The area of each leaf blade was determined with a planimeter just after it was removed. The average leaf area of the leaves of the first check was taken as the average leaf area, at the time of wounding, of each of the remaining members of the set. The leaf area removed in wounding was based upon these figures.

The types of wounding were carried out simultaneously—i.e., one plant in each consecutive pot of the set was wounded before the next plant in the first pot. This precaution was probably not necessary, yet it gave greater uniformity to the procedure. The types of wounding were as follows:

- o. Control (second check).
1. One leaf blade was removed.
2. The equivalent of one leaf blade was removed by removing half of each leaf. Standing facing the apex of the leaf, the right side of each blade was removed by cutting adjacent to the midrib with a scalpel.
3. The equivalent of one blade was removed by cutting out disks with a cork borer. Each disk had an area of one square centimeter and was taken as much as possible from between the veins.

4. One-fourth of the leaf area was removed by cutting away one-fourth of the leaf. Standing facing the apex of the leaf, the right basal quarter was removed from one and the right tip quarter from the other.

5. One-fourth the area of each leaf was removed with a cork borer delivering a disk of one square centimeter in area.

6. One-fourth the area of each leaf was removed with a cork borer delivering a disk of one-fourth of a square centimeter in area.

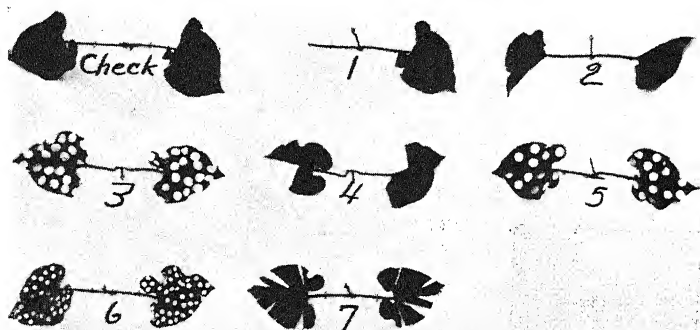


Fig. 1. Types of wounding.

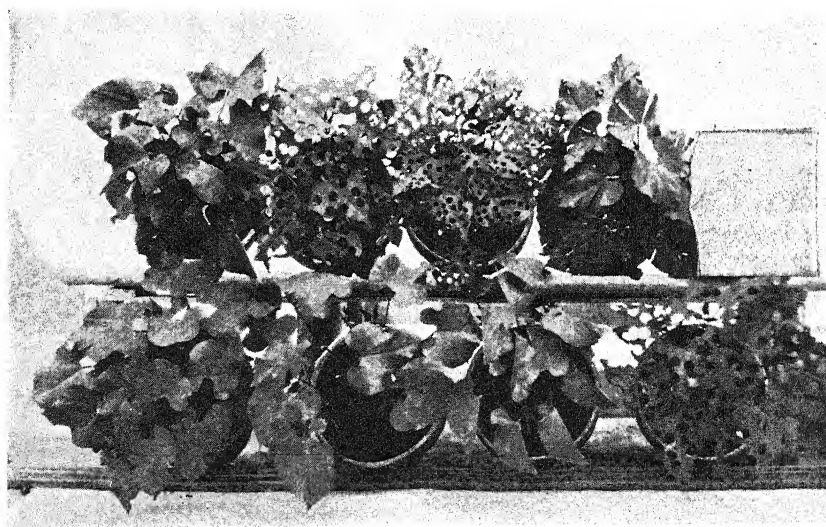


Fig. 2. Appearance of plants immediately after wounding.

7. The leaf area was not reduced but the leaves were slashed three times on each side. The slashes were made between the larger veins and extended practically to the midrib.

TREATMENT AFTER WOUNDING

The pots were equally spaced upon the benches. In order to maintain more uniform conditions within a set, the pots were rotated approximately every third day until the plants had reached the flower bud stage and were too large to handle. Water was added according to what appeared to be the need of the plants. The amount varied for each pot.

Since the sand did not contain sufficient mineral nutrients, the deficit was made up by the addition of a modified Knop's nutrient solution. The amount and the time of the addition were the same for each pot in a group but varied for the different groups. The amount used will be stated as each group is studied.

EXPERIMENTAL DATA

Well's Red Kidney (K) and Early Valentine (V) beans were used. Most of the data obtained were for the Red Kidney beans. However, group V-I will be substituted for group K-I, since two of the five sets of group K-I were burned while being brought to an air-dry condition. Statistical treatment of the data for the fresh weights of the two groups indicated comparable results.

The fresh and dry weights of the leaves, stems, and roots of the first check were also obtained when it was harvested at the time of wounding. These weights were taken as the weights of the corresponding parts of the seedlings for each member of the set at the time of wounding.

The fresh and dry weights of the leaf portions removed were obtained for each type of wounding separately. Each of these weights was subtracted in turn from that of the corresponding fresh and dry weights of the leaves of the first check. The differences represent the fresh and dry weights of the green leaf material remaining on each type immediately after wounding.

These latter weights were in turn subtracted from the weights of the leaves of the same type after harvest. The differences in weight represent the actual increase in weight of leaf material for each member of the set from time of wounding until harvest. Since the stems and roots of the wounded plants were not altered, the fresh and dry weights of the corresponding parts of the first check were subtracted from those of the final yields to get the increase in weight or yield of these parts from the time of wounding until harvest.

Plants were harvested at different stages of development. One group was harvested at the time of the opening of the first flower, another just prior to full bloom, a third just after full bloom, and a fourth when the pods were well formed. For convenience, the latter stage is designated as the "mature stage." Data for group V-I, harvested at "maturity," and for group K-III, harvested just prior to full bloom, will be given.

Group V-I was planted on July 29, 1929, and consisted of five sets. A set contained nine pots of eight plants each—one pot each for the first and

TABLE I. *Summary of fresh and dry weight gains for group V-I*
Fresh weights

	1	2	3	4	5	6	7
	One leaf removed	Half each leaf removed en bloc	Half each leaf removed (disks 1 sq. cm.)	One-fourth each leaf removed en bloc	One-fourth each leaf removed (disks 1 sq. cm.)	One-fourth each leaf removed (disks 1/4 sq. cm.)	Slash
Average	292.55 ± 12.30	276.73 ± 12.87	274.95 ± 10.05	292.78 ± 9.02	298.20 ± 12.90	287.86 ± 10.14	275.02 ± 19.52
D/P.E.	.32	.99	1.27	.02	.35	.34	.81
Second av.		305.97	285.97				300.49
<i>Dry weights</i>							
Average	34.10 ± .76	32.16 ± .98	33.44 ± 1.07	34.83 ± 1.34	34.78 ± .94	34.12 ± 1.34	32.21 ± 1.04
D/P.E.	1.27	1.56	.50	.47	.56	.01	1.47
Second av.		35.37	35.06				34.83

second checks and the seven types of wounding. The first set was wounded on August 8, sets 2 and 3 on August 9, and sets 4 and 5 on August 10. One hundred cubic centimeters of a modified Knop's nutrient solution were added on the following dates: August 1, 10, 13, 16, 19, 22, 25, 28, and 31. The plants were harvested on September 16 and 17. The average gain in the fresh and dry weights of the five sets constituting group V-I is shown in table 1. Since there were five pots containing eight plants each for the check and for each type of wounding, the average gain shown was obtained by dividing the total gain in each case by five.

The difference divided by its probable error (D over $P.E.$) is shown for each type. When 3.2 or greater, it is taken to indicate significance. Two averages and two differences divided by their probable errors are given for each type when necessary for clarity. The first average is derived from the sum of the same types in a group, regardless of the yield or condition of the plants at harvest. The second average is derived from the sum of the same types which to all appearances were normal and healthy when harvested. Low average yields in both the fresh and dry condition are shown for types two, three, and seven. In these types the root systems were in poor condition at harvest. In general, it appeared that the decrease in yield paralleled the condition of the roots.

The second averages are shown only for the types mentioned. Since the difference between the average yield of these types and that of the check is so small compared to the probable error of the check, it is obvious that no significant difference in yield is indicated. The data show that when the plants were grown to maturity and the total gain or yield was considered, no type of wounding is responsible for a significant gain or loss as reflected by the average fresh and dry weights.

Since the average total increase in yield of the plants for any type of wounding is not significantly different from that of the check, it remains to be seen if this holds true for the parts considered separately. The average fresh weight gains of the leaves, fruit, stems, and roots of the five sets constituting group V-I are shown in table 2.

The average yield of leaves for any type of wounding was larger than that of the check. If the average yield of the check were considered as 100 per cent, then the average yield of the different types of wounding would range from 100.85 to 109.97 per cent for the first average and from 100.85 to 113.96 per cent for the second average. Although the average yield was consistently greater, in no case was it significant.

The average yield of fruit was less for any type of wounding, with the exception of type five, where one-fourth the leaf area was removed, than that of the check. Considered upon the percentage basis, the yields ranged from 85.51 to 104.94 per cent for the first and from 88.78 to 104.94 per cent for the second average; yet there was no significant gain or loss in the average yield.

TABLE 2. *Fresh weight gains of the leaves, fruit, stems, and roots of group V-1*

	Check	1	2	3	4	5	6	7
		One leaf removed	Half each leaf removed en bloc	Half each leaf removed (disks 1 sq. cm.)	One-fourth each leaf removed en bloc	One-fourth each leaf removed (disks 1 sq. cm.)	One-fourth each leaf removed (disks 1/4 sq. cm.)	Slash
<i>Leaves</i>								
Average	79.38 ± 3.90	87.29 ± 3.14	80.07 ± 4.11	82.77 ± 2.33	86.35 ± 2.07	80.05 ± 4.55	80.05 ± 3.11	83.71 ± 5.39
D/P.E.		1.58	.14	.75	1.58	.05	.13	.65
Second av.			87.16	85.32				91.00
<i>Fruit</i>								
Average	74.28 ± 4.86	73.40 ± 2.54	71.28 ± 4.94	63.52 ± 3.93	69.35 ± 5.49	77.95 ± 4.17	73.12 ± 6.74	66.86 ± 6.72
D/P.E.		.16	.43	1.72	.67	.57	.03	.90
Second av.			82.63	65.95				73.38
<i>Stems</i>								
Average	60.46 ± 2.47	63.18 ± 2.88	56.84 ± 2.12	60.54 ± 2.83	61.89 ± 1.53	62.07 ± 3.20	58.35 ± 2.40	56.00 ± 3.35
D/P.E.		.72	1.11	.02	.49	1.64	.61	1.07
Second av.			61.05	63.16				60.79
<i>Roots</i>								
Average	78.42 ± 4.84	73.68 ± 1.97	68.54 ± 3.36	68.60 ± 2.87	76.00 ± 1.88	77.60 ± 4.77	76.34 ± 1.38	68.46 ± 5.10
D/P.E.		.91	1.68	1.75	.47	.12	.42	1.42
Second av.			74.46	71.04				75.33

The stems show less variation in the average yield. The range is from 92.62 to 104.49 per cent for the first and from 96.51 to 104.49 for the second average.

Partial defoliation decreased the average yield of roots in all cases below that of the check. The percentages ranged from 81.28 to 98.83 per cent for the first average and from 90.56 to 98.83 per cent for the second average. The removal of one-half the leaf area as in types one, two, and three reduced the root growth on the average more than the removal of one-fourth the leaf area as in types four, five, and six. As with the leaves and stems, the average yield of the roots was not significantly reduced by any type of wounding.

The average yield, as expressed by the fresh weights, of the parts of the plants subjected to any type of wounding employed is comparable to that of the corresponding part of the check. However, it is revealed that in all cases of wounding the average yield of leaves is higher and the average yield of roots is lower.

The average dry weights of the leaves, fruit, stems, and roots for group V-I are not shown, but they are in statistical agreement with the average fresh weights of the corresponding parts. The data are in accord in that no type of wounding gives a significant increase or decrease in yield above or below that of the check when the plant is considered as a whole or when the parts are considered separately. It should be borne in mind, however, that the wounded plants generally produced a higher yield of leaves and a lower yield of roots.

The seeds for group K-III were planted August 12, 1929. Sets K-12, K-13, and K-14, and K-15 and K-16 were wounded August 25, 26, and 27, respectively. The plants were a little more developed at the time of wounding than the plants of group V-I. One hundred cubic centimeters of a modified Knop's nutrient solution were added to each pot on August 22. This was repeated every third day until seven hundred cubic centimeters had been added to each pot. The first three sets were harvested September 23 and the remaining two sets the following day. The plants were harvested just prior to full bloom. The average gain in fresh and dry weight for each type of the five sets constituting group K-III is shown in table 3.

The fresh weight of the check shows a higher average yield than that resulting from any type of wounding employed. Considering the average yield of the check as one hundred per cent, the percentage yield shown for the various types of wounding ranges from 71.13 to 97.92 per cent for the first average and from 75.16 to 97.92 per cent for the second average. The removal of one-half the leaf area as in types one, two, and three gives a lower combined average yield than types four, five, and six, where one-fourth the leaf area was removed. The average gain for the former was 138.74 grams and for the latter 172.29 grams. The yields of types one and two only were significantly low, while the yield of type three was near the border line. This condition holds true for both averages.

TABLE 3. *Summary of fresh and dry weight gains for group K-III*
Fresh weights

	1	2	3	4	5	6	7
	One leaf removed	Half each leaf removed en bloc	Half each leaf removed (disks 1 sq. cm.)	One-fourth each leaf removed en bloc	One-fourth each leaf removed (disks 1 sq. cm.)	One-fourth each leaf removed (disks 1/4 sq. cm.)	Slash
Average	189.91 ± 9.49	138.41 ± 7.70	142.74 ± 11.61	185.96 ± 3.45	153.68 ± 7.04	177.22 ± 10.18	170.20 ± 3.83
D/P.E.	4.21	4.63	3.14	.39	3.06	1.24	1.91
Second av.	189.91 ± 9.49	142.56 ± 6.46	159.47 ± 3.52	185.96 ± 3.45	167.25 ± 5.70	177.22 ± 10.18	170.20 ± 3.83
D/P.E.	3.38	4.12	3.01	.39	2.05	1.25	1.92
<i>Dry weights</i>							
Average	23.90 ± .32	19.07 ± .32	20.09 ± 1.04	22.56 ± .36	21.34 ± .50	23.39 ± .46	22.52 ± .36
D/P.E.	6.94	10.64	3.40	2.74	4.30	.90	2.82
Second av.	23.90 ± .32	19.50 ± .34	21.56 ± .40	22.56 ± .36	22.11 ± .47	23.39 ± .46	22.52 ± .36
D/P.E.	10.32	9.41	4.52	2.74	3.14	.90	2.82

The yields as expressed by the dry weights are in agreement with those for the fresh weights with the exception of types three and five. The yield as indicated by the fresh weights for both types was near significance, while the figures for the dry weights indicate significantly low yields for type three in both averages and for type five in the first average. The differences in yield as indicated by the fresh and dry weights for these types are not so pronounced, since a slight shift in values would account for the differences shown.

The average fresh-weight gains of the parts for group K-III are shown in table 4. The percentage the yield of the leaves of the wounded plants is of the yield of the leaves of the check plants varies from 79.22 to 103.69 per cent for the first average and from 82.38 to 103.69 per cent for the second average. The demarcation in the combined average yields between the types having one-half and those having one-fourth the leaf area removed is not so marked as it was for the total yields, being 59.34 and 68.57 grams, respectively. Though the average increase in weight in the first case is more than 15 per cent lower than that of the check, neither shows a significantly lower average yield. The average yield of the stems in any case of wounding was more depressed than that of the leaves, and the separation upon the basis of the amount of leaf surface removed in wounding is more marked. The average fresh- and dry-weight gains of the stems for types one and two are shown to be significantly low.

The effect of wounding upon the yield of the roots was more pronounced. The removal of one-half the leaf area by any method gave a lower yield than did the removal of one-fourth the leaf area. The increase in yield of the former is approximately 80 per cent of the latter and about 70 per cent of that of the check. The average gain in yield was significantly low where one-half the leaf area was removed, but this was not the case where one-fourth the leaf area was removed.

When one-half the leaf area was removed in the form of one leaf or one-half of each leaf or as disks of one square centimeter in area, as in types one, two, and three, respectively, and the plants were harvested just prior to full bloom, the average yield of leaves was not significantly low for any type of wounding in comparison with that of the check, though in each case it was depressed. The average yield of both stems and roots is shown to be significantly low for types one and two, but only that of the roots for type three. The average yield of stems for the latter type was shown to be significantly low only by the dry-weight data.

Where one-fourth the leaf area was removed en bloc or as disks one square centimeter or one-fourth square centimeter in area, as in types four, five, and six, there was no clear-cut significantly low yield in comparison with that of the check for the leaves, stems, or roots, though the average yield of the stems in type five was in the border range of significance. Generally the yields of those types having the same leaf area removed were more comparable to

TABLE 4. *Fresh-weight gains of the leaves, stems, and roots of group K-III*

	I		2		3		4		5		6		7	
	Check	One leaf removed	Half each leaf removed en bloc	Half each leaf removed (disks 1 sq. cm.)	One-fourth each leaf removed en bloc	One-fourth each leaf removed (disks 1 sq. cm.)	One-fourth each leaf removed (disks 1 sq. cm.)	One-fourth each leaf removed (disks 1 sq. cm.)	One-fourth each leaf removed (disks 1 sq. cm.)	One-fourth each leaf removed (disks 1 sq. cm.)	One-fourth each leaf removed (disks 1 sq. cm.)	One-fourth each leaf removed (disks 1 sq. cm.)	One-fourth each leaf removed (disks 1 sq. cm.)	Slash
<i>Leaves</i>														
Average	71.47 ± 5.30	62.16 ± 3.37	56.62 ± 3.12	59.24 ± 4.94	74.11 ± 1.50	61.71 ± 3.01	69.88 ± .81	66.23 ± 2.06						
D/P.E.		1.48	2.42	1.69	.48	1.60	.30	.92						
Second av.	71.47 ± 5.30	68.75 ± 1.86	58.88 ± 3.51	66.43 ± 1.20	74.11 ± 1.50	68.46 ± 2.27	69.88 ± .81	66.23 ± 2.06						
D/P.E.		.48	1.98	.93	.48	.52	.30	.92						
<i>Stems</i>														
Average	55.07 ± 2.66	39.35 ± 2.03	41.39 ± 1.88	46.41 ± 3.42	51.71 ± 1.26	45.65 ± 1.36	53.85 ± 2.63	46.91 ± 2.13						
D/P.E.		4.67	4.27	2.00	1.14	3.16	.33	.39						
Second av.	55.07 ± 2.66	43.34 ± 1.06	42.14 ± 2.34	51.41 ± .80	51.71 ± 1.26	48.47 ± .75	53.85 ± 2.63	46.91 ± 2.13						
D/P.E.		4.09	3.65	1.32	1.14	2.39	.33	.39						
<i>Roots</i>														
Average	63.37 ± 2.64	36.89 ± 3.62	37.07 ± 3.07	37.09 ± 3.43	60.13 ± 4.32	46.31 ± 4.86	53.49 ± 2.36	57.15 ± 4.78						
D/P.E.		5.01	6.49	6.07	.64	3.68	2.70	1.13						
Second av.	63.37 ± 2.64	44.25 ± .49	41.54 ± .78	41.64 ± 1.98	60.13 ± 4.32	50.62 ± 3.89	53.49 ± 2.36	57.15 ± 4.78						
D/P.E.		10.85	7.93	6.58	.64	2.90	2.70	1.13						

each other than to those having different leaf areas removed. This leads to the suggestion that the amount of tissue removed was more important than the shape of the leaf part removed.

The data show that wounding changes the relationship between the rates of growth of the leaves, stems, and roots. The relative rate of growth of the leaves is increased at first, while that of the stems and roots is retarded. As the leaf yield of the wounded plants approaches that of the check, the relative rates of growth of the stems and roots increase in the order named. At the time of the harvest of the most mature plants, the yield of the roots had not completely overtaken the yield of the roots of the check plants.

Tables for groups K-II and K-IV are not given. The data for group K-II, which was harvested just after full bloom, gave results intermediate between those of groups V-I and K-III, while the results for group K-IV were in the same direction as those for K-III but more pronounced.

INFLUENCE OF WOUNDING UPON TOTAL YIELD

The total yield includes the actual weight at the time of harvest in addition to the weight of the material removed in wounding. Data were secured for the fresh and dry weights of group V-I and treated statistically. While not given, the data for the fresh and dry weights were in agreement and bring out the same general facts as the data for the gains in yield for that group. Data could be given showing the average gross yield and the average gross yield of the parts for the other groups, but since the results are comparable to those for the gains in weight in the same group, they are omitted.

FLOWER DATA

A study was made of the influence of wounding upon the time of the blooming period and upon the number of flowers produced. The new flowers were counted each morning. The data for group K-I are given in table 5. The figures given for each day for each type of wounding represent the total number of flowers produced by forty plants. The data indicate that all types of wounding except type seven delayed the blooming period from one to two days. It is further shown that all types of wounding with the exception of types one and five gave a greater total number of flowers than the check. The differences in these two cases are small. The average number of flowers produced daily was obtained by dividing the total number for each type by the number of blooming days for that type. The probable error of the check is 2.15. Since the greatest difference between the average number of flowers opening daily on the check plants and those wounded by any method is 3.39, it is obvious that there is no significant difference between the average number of flowers produced by the check and the wounded plants.

TABLE 5. *The number of new flowers opening daily on forty plants of each type of wounding in group K-I*

Dates		Check	I	Types of wounding						
				2	3	4	5	6	7	
Aug.	16	1	0	0	0	0	0	1	
	17	1	1	0	1	0	0	1	
	18	3	3	4	2	3	2	4	
	19	6	13	6	9	9	10	6	
	20	5	11	9	9	9	12	11	
	21	20	12	13	15	10	6	8	
	22	31	35	25	22	27	23	23	
	23	40	36	45	60	46	51	48	
	24	34	28	25	45	42	30	26	
	25	44	34	37	33	27	32	34	
	26	35	23	28	33	35	23	23	
	27	29	26	38	33	30	31	33	
	28	22	26	26	21	17	29	39	
	29	10	9	8	15	10	11	21	
30	16	13	11	16	15	14	20		
Sept.	31	2	6	6	3	4	7	2	
	1	11	14	13	12	14	19	15	
	2	9	11	17	15	15	13	14	
	3	3	13	19	6	12	12	19	
	4	9	11	16	10	12	5	10	
Totals		331	325	346	359	338	330	354	337
Average		. 16.55 ± 2.15	17.10	19.22	19.94	17.79	18.33	19.66	16.85	

WATER RELATIONS

The percentage of water varies not only with the state of turgor but also with the stage of development and the part of the plant under consideration. Data are given in table 6 for group V-I, showing the percentage of water in the leaves, fruits, stems, and roots as well as in the whole plant at the time of wounding and at the final harvest.

TABLE 6. *Percentage of water in the leaves, stems, roots, and fruit of bean plants at different ages*

Age in days	Leaves	Stems	Roots	Fruit	Whole plant
13	87.99	91.61	93.99		91.42
51	87.51	84.02	92.54	92.87	88.54

There is a higher percentage of water in the stems of the younger plants than in the leaves, while the reverse is true of the mature plants. The percentage of water in the leaves of the more mature plants was 99.45 per cent of the percentage of water in the leaves of the younger plants. Similar percentages for the stems and roots were 91.71 and 98.46 respectively. Taken as a whole the percentage of water in the maturer plants was 96.85 per cent of the percentage of water in the younger plants.

GENERAL DISCUSSION

Since the wounding consisted largely of the removal of portions of the initial pair of leaves, the question arises as to how the efficiency of the remaining portions was influenced. According to Blackman and Matthaei (1901), no healing reaction follows after a clean cut is made with a sharp knife through a cherry laurel leaf.

They state that the cells cut through die in addition to some three layers of cells, but never a sufficient number to produce a brown edge visible to the naked eye. With respect to beans, the brown edge was visible in many cases. However, the area involved was relatively negligible in comparison to the experimental error involved in the removal of the desired amount of tissue.

It was not always possible to avoid injury to some of the larger veins when the calculated amount of leaf surface was removed. Where this did occur, no additional effects could be detected. Wylie (1927) states in this connection that common mesophytic leaves may carry through their minor venation a conduction overload of several hundred per cent in addition to the traumatic water loss.

The increase in size of the first pair of leaves after wounding was determined in a few cases in groups K-II and K-III. The number of measurements was too limited for conclusive data, but the results obtained indicate that when one or a part of both of the initial pair of leaves is removed, there is a tendency for the remaining leaf or parts of leaves to grow to a larger size. A sudden change in any essential factor upsets the established balance of the plant. It is conceivable that when the leaf area was suddenly reduced, relatively more mineral nutrients and water were available for the remaining leaf area, since the absorbing system had not been altered.

A higher turgor is conducive to increased rate as well as to a greater total amount of growth. If such an unbalanced condition is conducive to a more rapid and a greater growth in the first leaves, it is reasonable to expect the same response from the new leaves, though in a decreasing degree as the original balance is approached. After the original balance is restored, no further increase in the size of the new leaves on the wounded plants over the size of comparable leaves on the check plants could be expected. The increase in the size of the leaves of the wounded plants probably accounts for the greater yield of leaves for those plants, as indicated in group V-I.

The yield of fruit was lower for all types of wounding except type 5. However, in no case was it significantly low. It is probable that if the same proportions of the leaf area had been removed at the beginning of the blooming period, the results would have been different, since the time for adjustment and replacement of leaf area would have been shorter. According to Culpepper and Magoon (1930) in work on corn, the yields as shown by the size of ears and numbers of kernels were most seriously affected when the plants were defoliated at the time of silking.

The data throw some light upon the relative distribution of growth be-

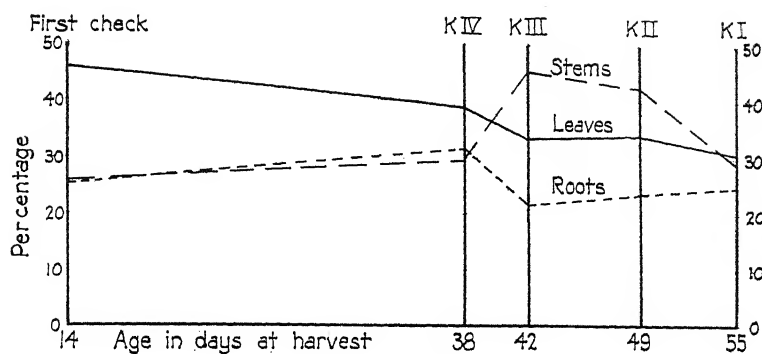


Figure 3. Check.

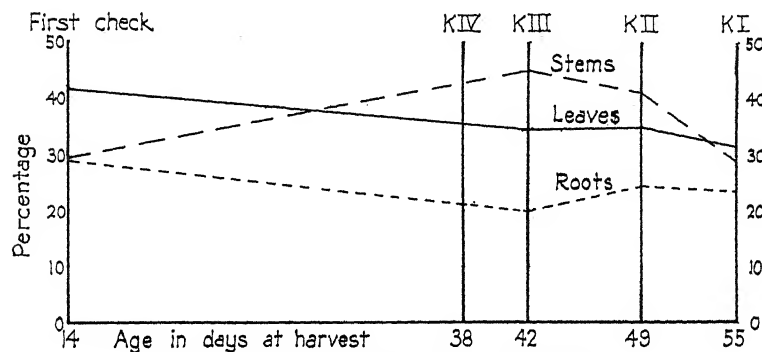


Figure 4. One fourth leaf area removed.

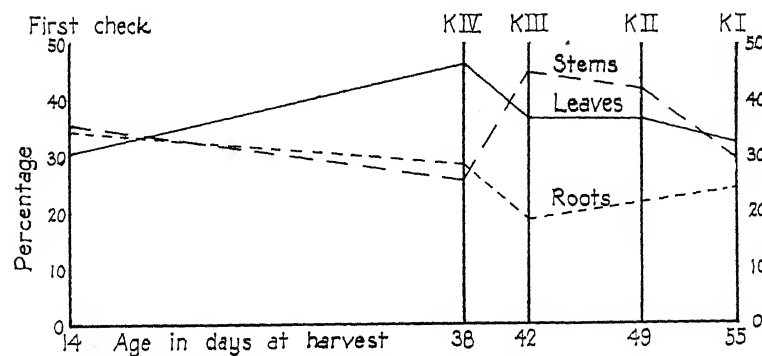


Figure 5. One half leaf area removed

Fig. 3, 4, 5. Gain in the yield of leaves, stems, and roots expressed as a percentage of the total gain at different harvest dates.

tween the leaves, stems, and roots at the various stages of development of the plants. Although the data are not strictly comparable, since the groups were planted at different dates and had slightly different amounts of mineral nutrients, it is thought that they are worth consideration. Another irregularity is that the percentages for group K-I are based upon the average of only three sets. Some of the types within these sets were not in good condition at harvest and may account for some of the irregularities of this group. The groups were harvested at the following intervals from the date of planting: The first check approximately fourteen days; at the beginning of the blooming period, thirty-eight days; just prior to full bloom, forty-two days; just after full bloom, forty-nine days; and at maturity, or fifty-five days. The first average is considered for the dry weights only.

Since the variation between the average yields of those types having the same leaf area removed was generally less than that between those types having different leaf areas removed, this was used as a basis for grouping the data. Consequently, the average for types one, two, and three, where one-half the leaf area was removed, and that for types four, five, and six, where one-fourth the leaf area was removed, constitute two sets of data. As no tissue was removed in the wounding of type seven, it was not included, though the yields of this type compare favorably with those where one-fourth the leaf area was removed. The data represent the gain in yield of the parts expressed as a percentage of the total gain and are shown in the form of graphs in the figures which follow. Figure 3 represents the check, figure 4 the average of types 4, 5, and 6, and figure 5 the average of types 1, 2, and 3.

Fourteen days after planting, the leaves, stems, and roots represent approximately 47, 27, and 26 per cent, respectively, of the total dry weight as shown in figure 3. Twenty-four days later the relative percentage of leaf yield has decreased; yet it is still higher than that of the stems or roots. During this period the roots increase relatively more than the stems and constitute a greater percentage of the whole. From the inception of the blooming period to a period just prior to full bloom a great shift in the percentage yield of the parts occurs. There is a sudden drop for the leaves and roots and a corresponding increase for the stems. The percentages for the parts given in the same order as above are now roughly 33, 45, and 22. The next period shows no change in the relative positions and little variation in the relative percentages, although both leaves and roots make slight relative gains, while the stems show a decline. When the fruit sets and begins to grow, there is a marked decline in the percentage yield of stems accompanied by a smaller decline for the leaves. Even under these conditions the yield of the roots expressed as a percentage continues to increase in relative value.

Figure 4 is not strictly comparable to the other graphs for the period from the fourteenth to the forty-second day, since no plants were harvested on the thirty-eighth day. The figure shows a decrease in the relative percentages of yield for both the leaves and roots and an increase for the stems until the

forty-second day. If a harvest had been made on the thirty-eighth day, it is likely that the differences would not have been so marked between the two periods and that they would have been more marked from the thirty-eighth to the forty-second day. After this period figure 4 is comparable to the check graph, though there are small differences in the degree of the trend of gain in yield.

The greatest contrast between the relative percentage yield of the check and that of the most severely wounded plants, as shown in figure 5, occurs between the fourteenth and thirty-eighth days. The check plants show a decrease in the relative percentage yield of leaves, while there is a marked increase for that of the plants where one-half the leaf area was removed. While the relative percentage yield of the stems and roots of the check increased slightly during this period, there was a decrease in the percentage yield of these parts of the wounded plants. After the thirty-eighth day the general increase or decrease in the percentage yield of the parts for the treated and untreated plants was of the same order, though the changes for the wounded plants were a little more pronounced.

The similarity of the data for the total gains and the gain in yield of the parts and the total gross yield and the gross yield of the parts is so striking that no detailed study of the data for the latter is given.

A marked change in the relative position of the parts occurs between the thirty-eighth and forty-second days. It may be noted in this connection that group K-IV came into flower three days later than group K-III.

In order to facilitate direct comparison of the relative gains in grams of the parts, as expressed by the dry weights, at the different times of harvest, figures 6, 7, and 8 are shown. The check is represented by figure 6, while the types of wounding were grouped in figures 7 and 8 as in figures 4 and 5. These figures show that even though the yield of a part expressed as a percentage of the total yield may change position in relative value as compared to another part, it usually continues to increase in size to "maturity." Figures 9, 10, and 11 contain the same data as figures 6, 7, and 8, but the data are rearranged for more direct comparison.

The above data are based upon the results obtained from plants grown in six-inch pots. According to Crist and Stout (1929), the ratio between the top and root weights varies with the size of the container in which the plants are grown. These authors state that the plants they used grew to a greater size in larger containers, with both leaves and stems increasing in weight and each of these making a greater relative growth than the roots.

The relative percentage increase in the yield of the roots of the wounded plants was usually lower during the earlier stages of development than for either the leaves or stems. This condition prevailed until about the beginning of the blooming period, when apparently the rate of root growth became accelerated to a degree slightly above that of the check plants. The plants of group V-I were the most mature when harvested. The root yield of the

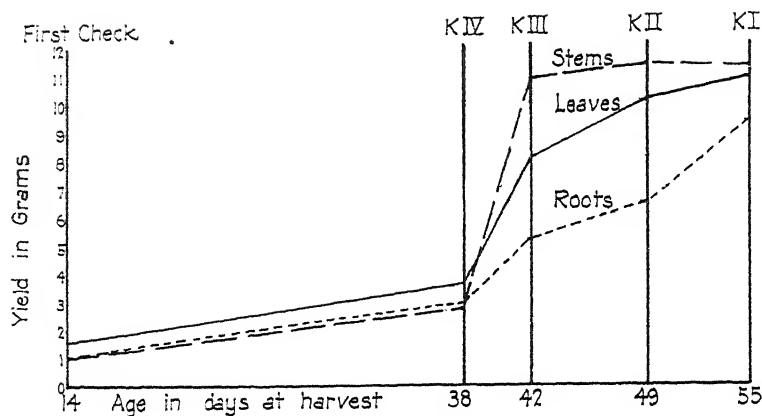


Figure 6. Check.

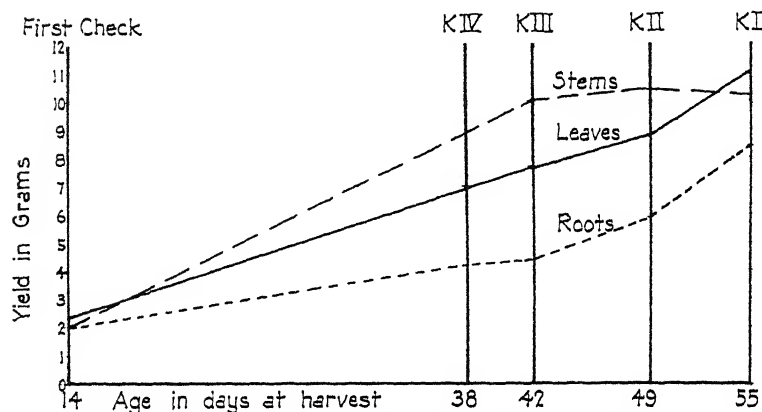


Figure 7. One fourth leaf area removed

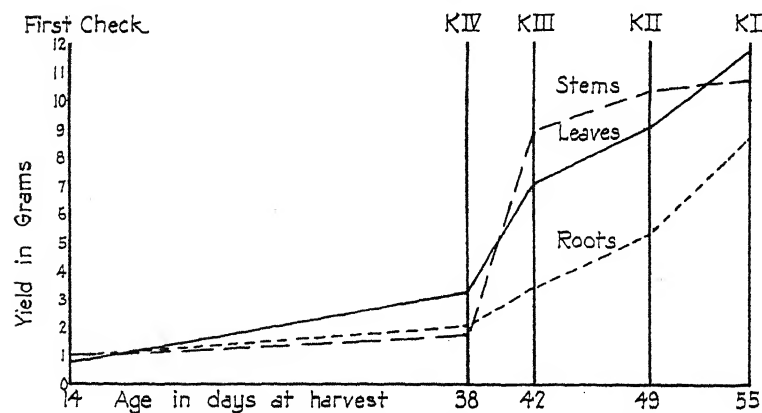


Figure 8. One half leaf area removed

Fig. 6, 7, 8. Average yield in grams of the leaves, stems, and roots at different harvest dates (dry weights).

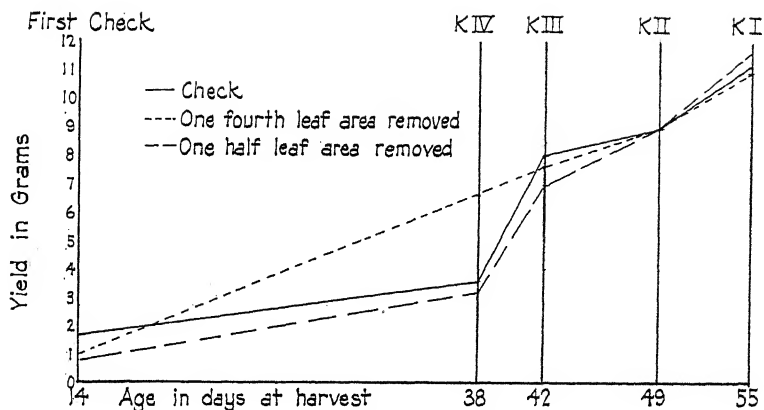


Figure 9. Leaves

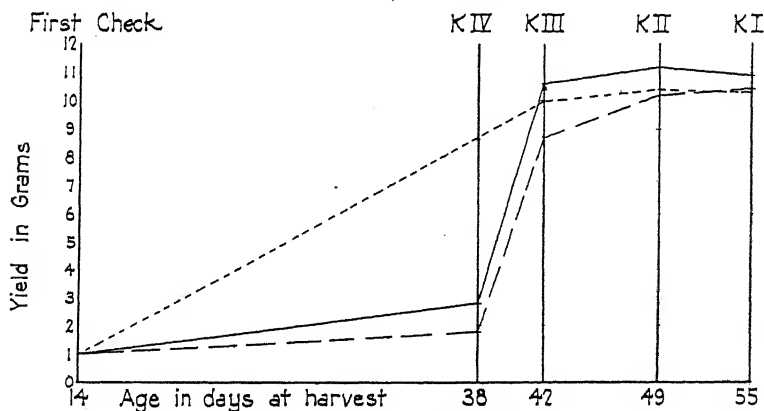


Figure 10. Stems

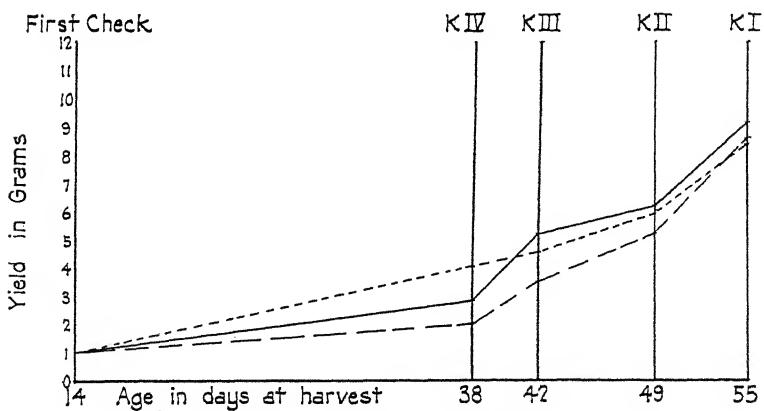


Figure 11. Roots

Fig. 9, 10, 11. Rearrangement of data from fig. 6, 7, and 8 to facilitate comparison of gains.

wounded plants of this group was still a little lower than that of the corresponding check. Although these plants were designated as mature at harvest, the leaves and pods were still green and possibly some growth might have taken place later. Therefore no data are available to show whether the root yield was permanently influenced or whether it would have eventually become equal to that of the check had the harvest been later.

The disks were removed to simulate defoliation as a result of insect injury. The data reveal that unless there is some effect other than mechanical removal of leaf area, at least one-half of the leaf area can be removed by insects, if taken before the plants have more than two leaves, without a subsequent significant reduction in yield as a result of such injury. The same would probably hold true for leaf-spot diseases provided it were only a matter of reducing the photosynthetic area. But since the cells of the susceptible are killed in many cases in advance of the mycelium, it suggests the presence of a toxic substance—the action of which might not be comparable to mechanical injury. The slashing was done with a sharp scalpel and differs from the tear and bruise as a result of hail. The slashing as carried out was not accompanied by a significantly low yield.

No significant difference between the yields of different types of wounding is revealed by the data where the same amount of leaf area was removed and the plants were allowed to reach "maturity," as, for example, in types one, two, and three, where one-half of the leaf area was removed, and for types four, five, and six, where one-fourth the leaf was removed. However, in the earlier stages of development, the heavier types of wounding gave a more marked reduction in yield than did the lighter types of wounding. The differences became progressively smaller as the plants approached "maturity."

TABLE 7. *A study of the efficiency of the leaves of group V-I*

	Average total leaf yield	Actual average increase in total yield	Calculated increase	Per cent efficiency
Check	11.29	34.10	—	100
First division. Types 1, 2, and 3 (one-half leaf area removed). First average	11.19	33.65	33.79	99.59
Second division. Types 4, 5, and 6 (one-fourth leaf area removed) ...	11.50	34.58	34.73	99.57

A method for arriving at an approximate measure of the efficiency of the leaves of the wounded plants as compared to that of the check plants is given below. The data shown in the tables are derived from the dry weights of group V-I and K-III. The leaves being primarily the photosynthetic organs, the average total yield of leaves at the time of harvest is considered

in relation to the average total gain in yield. The types of wounding have again been divided into two groups, the basis of division being the amount of leaf tissue removed. Only the averages for the check and for each division are considered in the tables. The data for group V-I are given in table 7.

The average total leaf yield of the check was 11.29 grams and the actual average increase in total yield was 34.10 grams. For each gram of leaf material at the final harvest there was a final increase in total yield of 3.02 grams. A yield of this ratio will be considered 100 per cent efficient. Now, if the average yield of the wounded plants of each division is multiplied by the factor 3.02, it will give the figures shown in the third column; these represent the yields of the plants in divisions one and two provided they had produced at the same ratio as the check.

The actual average increase in total yield for the check and each division is shown in column two. The actual increase divided by the calculated increase gives the percentage of efficiency found in the fourth column. The percentages indicate little, if any, difference in the efficiency of the leaves when the efficiency is determined by the above method.

The data for group K-III are given in table 8.

TABLE 8. *A study of the efficiency of the leaves of group K-III*

	Average total leaf yield	Actual average increase in total yield	Calculated increase	Per cent efficiency
Check	10.05	23.90	—	100
First division. Types 1, 2, and 3 (one-half leaf area removed). First average	8.20	19.10	19.52	97.85
Second division. Types 4, 5, and 6 (one-fourth leaf area removed). First average	9.45	22.43	22.49	99.73

For each gram of total leaf yield of the check there was a final increase in total yield of 2.38 grams. The calculated yields are shown in the third column and the per cent efficiency in the last column. The figures show an efficiency of 97.85 and 99.73 per cent where one-half and one-fourth the initial leaf areas were removed. In divisions one and two, where one-half and one-fourth the initial leaf areas were removed, the total yield of leaves as well as the total gain in yield was lower than that of the check. In this case the more severe the wounding, the lower were the yields and the percentage of efficiency. It has been shown that one result of wounding was a change in relationship between the rates of growth of the leaves, stems, and roots. At first the rate of leaf growth was accelerated and that of the stems and roots retarded. In case there has been the same amount of total growth and the

distribution of this growth so modified that a higher percentage occurs in the leaves, there will be a corresponding decrease in the per cent efficiency when it is determined by the method used. Thus the lower percentages shown for the efficiency of the leaves of the wounded plants may be more apparent than real.

SUMMARY

1. Bean plants were grown in sand contained in six-inch unglazed pots. There were eight plants in each pot.
2. The seed and sand were inoculated at the time of planting.
3. The wounding was restricted to the first pair of leaves and was carried out before the other leaves expanded.
4. The results showed that when the plants were allowed to reach "maturity," the difference between the gains in yield of the check plants and the gains of those plants subjected to any type of wounding was not significant. This was true for the total gain and for the gain of any part, as leaves, fruit, stems, or roots. However, the average yield was usually higher for the leaves and lower for the fruit and roots of the wounded plants than for the corresponding parts of the check.
5. The gross yield or that of any part of the mature plants subjected to any type of wounding was not significantly lower than that of the check plants taken as a whole or that of the corresponding parts considered separately.
6. If the plants were harvested just prior to full bloom, the difference between the total gain in yield of the check and that of those types of wounding where one-half the leaf area was removed was generally significantly low, while it was not for those types where one-fourth the leaf area was removed. When the gain in yield of the parts, as the leaves, stems, and roots, is considered, no type of wounding was responsible for a significantly lower yield of leaves. However, that of the stems and roots was generally significantly low when one-half the leaf area was removed but not when one-fourth the leaf area was removed.
7. When one-half the leaf area was removed, the plant responded by a marked increase in the relative rate of growth of the leaves and by a smaller decrease in the relative rate of growth of the stems and roots as compared to the untreated plants. The balance between the relative yields of all the parts was not completely restored at the time of harvest of the most mature plants.
8. When one-fourth the leaf area was removed, the tendency was to respond in growth in the same way as when one-half the leaf area was removed, but to a less marked degree.
9. The data indicate that the amount of leaf area removed was more important than the method of removal—i.e., whether it was removed en bloc or in the form of disks.
10. The removal of one leaf or one-half of each leaf by cutting along the

midrib tended to induce the remaining leaf or leaf halves to grow to a larger size than would have been the case without such treatment.

11. Wounding generally delayed the blooming period, from one to two days. When both leaves were removed, it was delayed from one to four days. The average number of flowers produced was not significantly changed as a result of wounding.

12. There was little variation between the percentages of water in the green leaves of the plants fourteen days old and in those of plants fifty-five days old. There was more variation in the percentage of water in the stems than in the leaves or roots.

The writer is indebted to Dr. O. F. Curtis for suggesting the problem and for advice and criticism in the interpretation of the data and in preparation of the manuscript; to Dr. H. H. Love for advice on statistical methods; and to Dr. H. C. Thompson for suggestions on the variations of beans.

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STUDIES IN *SAXIFRAGA*. III. *SAXIFRAGA*. SECTION
MICRANTHES

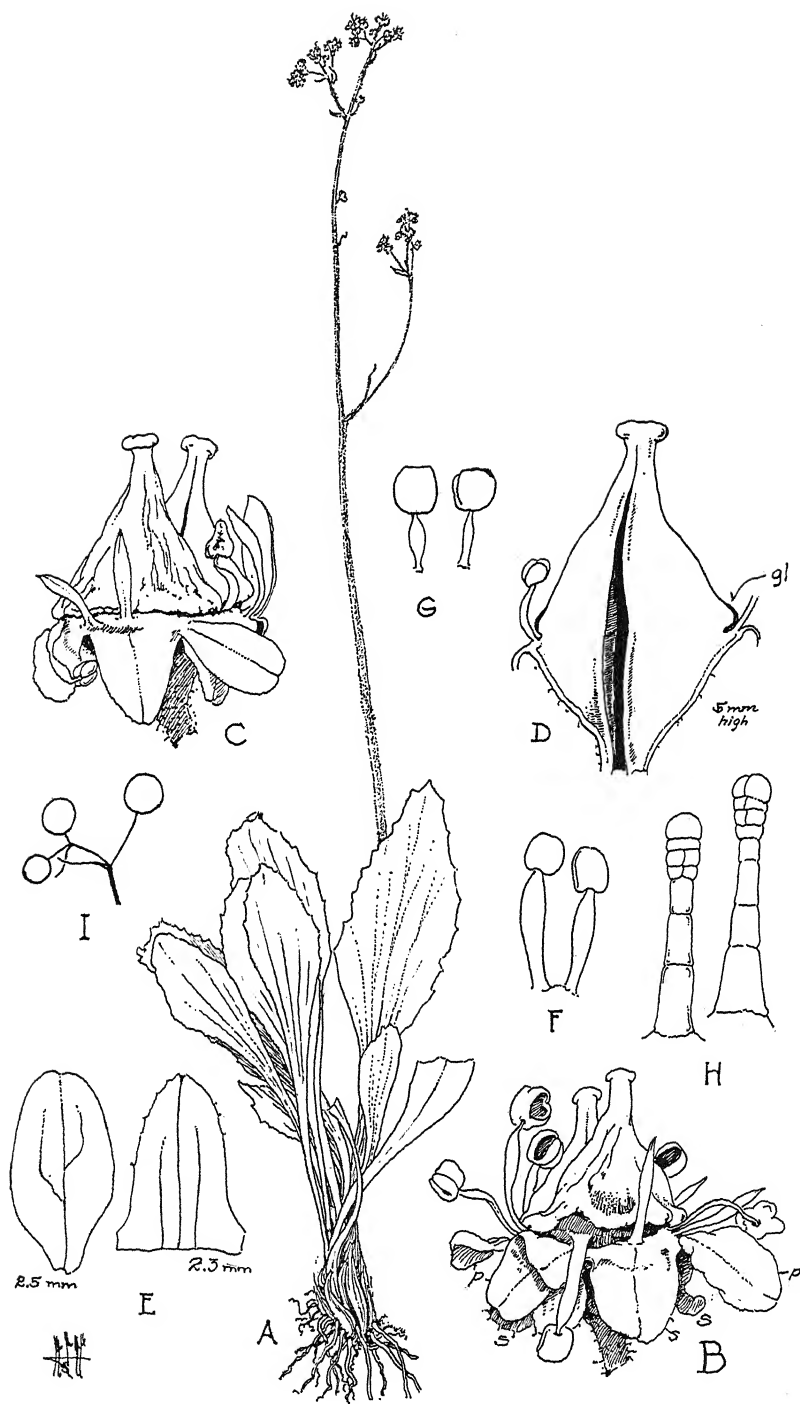
A. M. JOHNSON

(Received for publication February 2, 1933)

Saxifraga chelanensis sp. nov.

Perennis caudice brevis fibroso caule florifero scapiforme 24–30 cm. alto tota longitudine pilis glanduliferis brevibus dense obsita, folia basalia ascendentia tenuia plana 3–10 cm. longa lamina elliptica vel oblonga vel ovato-oblonga plus minusve remote dentata vel denticulata in petiolum longum sensim contracta, juvenulis infra obscure pubescentibus et margine ciliolatis adultis infra glabris vel subglabris, inflorescentiae rami pauci graciles adscendentes superne abbreviati prophyllis parvis linearibus instructis, flores pauci parvi sepala oblonga vel ovato-oblonga vel triangularia obtusa circa 1.5–3 mm. longa viridia 1–3-nervia in anthesi valde reflexa sparse glanduloso-ciliolata et obscure et irregulariter denticulata, petala alba parva sepalis subaequilonga vel longiora circa 2–3 mm. longa obtusa retusa late unguiculata uninervia nervo medio 1–2-ramoso, stamina filamenta clavata sepalis petalisque subaequilonga antherae rubiginosae, carpodia dua distincta circa 4–5 mm. longa ovarii hypanthio ad medium longitudinis adnatis disco valde lobato instructis in stilos crassos divergentes subito contractis stigmatibus discoideis. Fructum non vidi.

Plants perennial from a short fibrous oblique caudex; flowering stems 24–30 cm. high, densely glandular pubescent with short hairs throughout its entire length, branching above into an open, rather few-flowered cymose panicle inflorescence of a very few slender spreading ascending branches which are much shortened upwards, are subtended by small linear bracts, and terminate in small, loose, mostly 2–3-flowered, glomerules. Leaves basal, ascending-spreading up to 10 cm. long, flat and mostly very thin, the blades elliptic to oblong or ovate-oblong, obtuse, mostly remotely and shallowly dentate to serrate-dentate, or denticulate, when young glandular ciliate and obscurely glandular pubescent beneath but becoming glabrous or nearly so with age, all tapering more or less gradually into a long thin flat, basally sheathing, petiole. Flowers small; sepals broadly to narrowly oblong-ovate or oblong, more or less obscurely denticulate, and very sparsely or scatteringly minutely glandular ciliate, green and strongly reflexed at anthesis; petals small, up to 3 mm., white, about equalling or sometimes exceeding the sepals, but frequently varying considerably in size in the same flower, long, elliptic to elliptic-ovate, more or less rounded and notched at the apex and tapering abruptly into a broad claw at the base, the solitary midrib frequently with one or two short ascending lateral branches from the middle region; stamens 10, slightly exceeding the sepals at anthesis but elongating with age, the anthers deep to pale rose-red, becoming whitish with age, introrse, the filaments clavate but appearing subulate with age; carpels 2, distinct, up to 5 mm. in length; ovaries deeply adnate to the turbinate receptacle and expanded into a rather strongly but irregularly lobed gland immediately above the insertion of the stamens, and strongly contracted upwards into rather



JOHNSON: SAXIFRAGA

stout divergent styles which terminate in discoid stigmas. Ripe fruit not seen.

Specimens examined: J. William Thompson, 5979, wet slopes, 10 miles north of Entiat, Chelan County, Washington, April 18, 1931, *type*.

The most distinctive feature of this species is the clavate filaments with their rose-red anthers. Clavate filaments have not heretofore been seen by the writer in any other species of the Section *Micranthes*. The clavate character is not apparent in dry material but becomes clear on moistening. One flower showed 3 carpels present, and another had two supernumerary aborted carpels.

CASCADIA,¹ gen. nov.

Carpidia dua ad basim distincta, stilis brevibus attenuatis paulum divergentibus; ovaria fere ad $1/3$ longitudinis receptaculo turbinato immersa supra medium angusta glandula annularia cincta; semina oblonga seriatim distincte longitudinaliter pectinata; hypanthium altum attamen quam sepala brevius; sepala triangularia acuta; petala oblonga vel obovato-oblonga; staminum filamenta subulata hypanthii margine inserta.

Carpels two, distinct to the base; styles short, attenuate, slightly divergent; ovaries immersed for about $1/3$ of their length in a turbinate receptacle, and encircled above the middle by a narrow annular gland (disk); seeds oblong, distinctly pectinate in longitudinal rows; hypanthium deep but shorter than the sepals; sepals triangular, acute; petals oblong to obovate-oblong; filaments subulate, inserted on the margin of the hypanthium.

Cascadia Nuttallii (Small), comb. nov. *Saxifraga Nuttallii* Small. Bull. Torr. Bot. Club 23: 268, 1896. N. Am. Fl. 22: 126, 127, 1905. *Saxifraga elegans* Nutt. ex Torr. & Gray, Fl. N. Am. 1: 573, 1840, not *S. elegans* Sternb.

Plantae solitariae annuae, caule 4-40 cm. alto gracile simplici vel e medio ramoso inferne glabro, e medio superne pilis glanduliferis sparsissime obsito, folia parva plus minusve remota glabra, lamina elliptica vel ovato-elliptica ad apicem triloba, inflorescentiae laxae ejusdem rami tenues vel filiformia paucivel pluriflori, prophylla lanceolata sessilia vel brevissime petiolata, flores albi, sepala triangularia acuta divergentia persistentia, circa 2 mm. longa trinervia nervis lateralibus sub apice medio nervo nonnunquam confluentibus, petala

DESCRIPTION OF PLATE 1

Saxifraga chelanensis sp. nov. A, habit sketch. B, one of the larger flowers of a glomerule, with petals folded back, showing variations in the degree of clavateness of the filaments. C, a flower with an aborted stamen and rather short filaments. D, the ventral aspect of a carpel. E, a petal (left), and a sepal (right), showing shape and venation. F, two stamens from a flower before anthesis. G, two stamens from a very small flower bud. H, glanduliferous hairs from the stem. I, diagram of a glomerule, with the relative sizes of the flowers indicated.

¹ See further discussion in Amer. Jour. Bot. 14: 38-43, 1927, where published as *nomen nudum*.

circa 4 mm. longa, oblonga vel ovato-oblonga base brevissime unguiculata trinervia nervis lateralibus e medii nervi base exeuntibus et sub apicem medio nervo saepe confluentibus, staminum filamenta subulata hypanthii margine inserta, capsula circa 5 mm. longa carpidiis ex hypanthio circa 1-3 toti ipsorum longitudinis projectantibus, semina oblonga supra medium latiora dorsiventraliter paulum curvata et dorsaliter seriatim pectinata.

Plants solitary, annual; stems 4-40 cm. high, slender, simple or branched from the middle, glabrous below but sparsely clothed with glandular hairs above the middle. Leaves small, more or less remote, glabrous, the blades elliptic or ovate-elliptic, three-lobed or coarsely three-toothed at the apex, the middle lobe much the largest. Inflorescence lax, the branches slender or filiform, few to many-flowered; bracts foliaceous, lanceolate, sessile or very short-petiolate. Flowers white; sepals triangular, acute, divergent, persistent, about 2 mm. long, three-nerved, the lateral nerves sometimes confluent with the midnerve below the apex; petals about 4 mm. long, oblong or obovate-oblong, shortly unguiculate, three-nerved, the lateral nerves arising from near the base of the midnerve and often confluent with it at apex; stamens subulate, inserted on the margin of the hypanthium. Capsules about 5 mm. long, the carpels projecting above the hypanthium (or from the receptacle) for about $1/3$ of their length, the styles strongly divergent at maturity of the fruit; seeds oblong, broadest above the middle and slightly curved, and beset with longitudinal pectinate ridges.

Specimens examined: Nuttall. ex Herb. Elias Durand, 1866; Columbia River, on moist rocks by springs. Elihu Hall, 156, "Ore.," 1871. J. Howell, wet rocks, Milwaukie, Ore., 1877. W. C. Cusick, 1846, Cascade Mts., Quinn Co., Ore., 1887, *type*. J. C. Nelson, 1242, wet cliffs, Elk Rock, Ore., June 2, 1917. J. C. Nelson, 2112, on rocky cliff, Oregon City, May 4, 1918. J. William Thompson, 4977, Marion Co., Ore., "Under spray of Silver Creek Fall, 1000 feet." July 11, 1928.

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AN INSECT VECTOR OF THE FIJI DISEASE OF SUGAR CANE¹

GERARDO OFFIMARIA OCFEMIA

(Received for publication February 7, 1933)

In June, 1928, the writer first attempted to transmit the Fiji disease of sugar cane (*Saccharum officinarum* L.) by the use of insect vectors. The earlier trials to this end were made at Los Baños, P. I., at intervals during 1928-1930. The insects used were two species of aphid (*Aphis maidis* Fitch.² and *A. sacchari* Zehntner) and one coccid (*Trionymus sacchari* [Cockerell]). Some Fiji disease appeared in the experimental canes with each of the three insects, respectively. Although all of the checks remained healthy, these results were not considered really significant, since in all cases the experimental canes were transplanted to unprotected garden plots. Moreover, in all except the last trial two-node cuttings were used as "seed" canes. Inasmuch as Fiji disease occurred on adjacent non-experimental canes, it was concluded that the appearance of the Fiji disease as noted might be attributed to conditions or agencies other than the insects used experimentally.

In 1931³ it was possible to construct adequate insect-proof chambers, designed by the writer, which, with the properly guarded use of single-node cuttings, justify confidence in the experimental methods used in 1931-32 and the validity of conclusions reached. In these later experiments the leaf-hopper *Perkinsiella vastatrix* Breddin (Delphacidae) was tested as an agent of transmission.⁴

¹Contribution from the Experiment Station of the College of Agriculture, Los Baños, Laguna, Philippine Islands. Published with the approval of the Director.

²The writer is indebted to his colleague, Professor Leopoldo B. Uichanco, Head of the Department of Entomology, for the identification of all insects listed in this paper. He is also indebted to the Photographic Division of the Department of Soils for the photographs used in all of the illustrations that follow.

³Professor L. R. Jones, of the University of Wisconsin, visited our experimental fields in November, 1931, and gave us encouraging counsel. He has also kindly advised in the preparation of this report of progress.

⁴The first mention in print of the transmission of the Fiji disease of sugar cane by the leaf hopper *Perkinsiella vastatrix* Breddin appeared in a short note about the writer's work published in *The Philippine Agriculturist*, Volume 21, No. 5, October, 1932.

While the present paper was in press, an article by R. W. Montgomery and Arthur F. Bell appeared in *Queensland Bureau of Sugar Experiment Stations Division of Pathology Bulletin* 4: 5-28, 1933. The authors of the article report successful transmission of the Fiji disease of sugar cane in Australia by nymphs of the leaf hopper *Perkinsiella saccharicida* Kirk., the species that occurs in Australia and Hawaii but not in the Philippines.

[The JOURNAL for February (21: 55-112) was issued February 6, 1934.]

MATERIALS AND METHODS

Insect-proof cages

The two insect-proof cages used in the following experiments are illustrated in figure 1 of plate 1. Each consisted of a wooden framework 150 cm. long, 150 cm. wide, and 183 cm. high, opening by a narrow door and with panels on top and sides covered by 80-mesh brass wire gauze. The legs rested on concrete bases, the central portions of which were surrounded by a trough containing water with a layer of oil on top, a trap for ants and other crawling insects. The bottom of each cage was lined with galvanized iron, provided with drainage holes. For each experiment it was filled to a depth of 40 cm. with garden soil that had been steam-sterilized for two or more hours at 10 to 15 lbs. pressure. The cages were located in the open air, exposed to full sunlight.

Preparation of the cuttings

In all of the 1931-32 experiments, the sugar cane variety POJ 2878 was used, because, although highly resistant to the mosaic disease, it is susceptible to Fiji disease. Each selected cane stalk was examined with great care to make sure that leaves and sheaths were free from Fiji galls; also that all eyes were normal and free from fungous and insect attacks. It was then washed in running tap water, and the nodes were numbered consecutively from the base upward by means of the point of a clean, sharp scalpel. The stalk was then cut into sections, each about 50-60 cm. long, which were immersed in tap water in a large battery jar for from 24 to 48 hours. Each internode was then girdled 3-6 mm. deep (fig. 1), and the pieces were wrapped with wet filter paper or cheesecloth and stored in another large battery jar until eyes and roots started to grow. When such growth had developed shoots 1-5 cm. long with abundant roots, each section was divided into one-node cuttings by thrusting a clean sharp knife through the girdles around the internode.

These procedures, including the one-node cuttings, were followed as a precaution against the possibility of use in our experiments of Fiji-infected nodes or internodes. The writer is of the opinion that such soaking and storage as are above outlined would allow the infective principle of Fiji disease, were this present in any part, to diffuse throughout the section during the storage period and before the one-node cuttings were made. Stahl and Faris (1929), working with POJ cane (mosaic-resistant), showed that the mosaic virus is not distributed throughout the entire stalk of POJ as it is with susceptible varieties. Our own experiments with Fiji disease, on the other hand, seem to indicate that the infective principle is uniformly distributed throughout the infected stalk of POJ 2878. Thus far, seven trials of one-node cuttings made from seven different stalks of this variety, which had been infected with Fiji disease in the field, have shown that all the eyes produced diseased shoots.

Using the one-node cuttings, prepared as above, each cutting was planted

in a 24-cm. pot of steam-sterilized soil, which was then placed in its proper chamber. All even-numbered nodes were used as checks and placed in the same insect-proof check cage. The odd-numbered cuttings were used for Fiji transmission experiments and placed in the transmission cage.

Transfer of leaf-hoppers

The adults of the insect to be tested as vector were transferred by means of an aspirator. The model for the construction of this was kindly loaned the

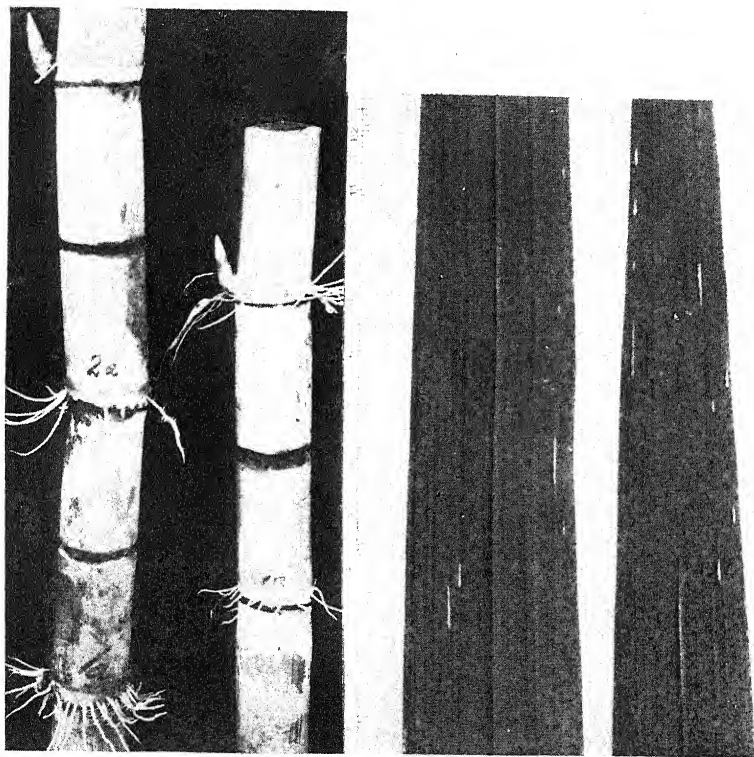


Fig. 1 (left). Stalk *a* of sugar cane POJ 2878 used in experiment 3 in 1932. The stalk was cut into two portions to facilitate soaking in water in a battery jar, and it shows the girdled internodes with numbers written above the nodes and the growing eyes and roots. The numbers written above the nodes are indistinct in the photographic reproduction; they are 1*a*, 2*a*, and 3*a* at the left, and 4*a* and 5*a* at the right—the numbering being from bottom to top in each case. The photograph was taken after the cuttings had been immersed in tap water for 48 hours and then wrapped with wet cheesecloth for seven days in order that the roots and eyes might develop. About 1/3 natural size.

Fig. 2 (right). The upper portion of the youngest expanded leaf of shoot from node 15 photographed on September 8, 1932, to show varying ages and sizes of galls of the Fiji disease. The leaf was cut into halves, and the parts were placed side by side to show the presence of very young galls as well as galls which are well developed. The strings of very young galls are not distinguishable in the photograph. The scale applies to figure 2.

writer by Dr. Leopoldo B. Uichanco. This aspirator operates on the same principle as that described and illustrated by Kunkel (1926, text figure 4), except that Dr. Uichanco's modification of the French aspirator, by means of a piece of silk bolting cloth, prevents the insects from entering the mouth of the operator by way of the rubber tubing. This piece of bolting cloth is fastened over the curved end of the glass tubing, to the opposite end of which the rubber tubing is attached. This modification also does away with Kunkel's insect holder, because once the insects are within the glass chamber of the aspirator they cannot escape from it.

TRANSMISSION EXPERIMENTS AND RESULTS

Experiment 1. The two insect-proof chambers contained potted cane plants that were about five months and twenty days of age. The potted canes came from one-node cuttings of a stalk of POJ 2878 cane secured from the Department of Agronomy of the College of Agriculture, December 16, 1931, and were planted on December 28. On May 24, 1932, the main stalk of each cane was removed to prevent the tops from pushing against the wire top of the cage and to induce tillering. The transmission chamber contained potted shoots from eyes 1, 3 (dead), 5, 7 (placed outdoors and discarded later), 9, 11, 13, 15. The control insect-proof chamber contained potted shoots from eyes 2, 4, 6, 8, 10, 12, 14, 16.

On June 17, 1932, an abundance of adult leaf-hoppers, *P. vastatrix*, were placed in the inoculation chamber. These came from individuals and their progeny which had been confined on Fiji-infected canes since March 9, 1932. No insects were introduced into the control chamber.

On August 5, 1932, after an incubation period of about 49 days, it was found that shoots 1, 13, and 15 had very young galls on the veins of the nether surface of the lamina of the leaves and on the midribs (fig. 2). On August 15, shoots 9 and 11 showed the disease, an incubation period of 59 days, and shoot 5 on August 23, an incubation period of 67 days. The checks of these shoots (the even-numbered) were all free from Fiji disease (pl. 1 fig. 2).

Experiment 2. This was a duplication of experiment 1 in all details except that another stalk of cane POJ 2878 was used. All operations in the two experiments were performed on the same dates. From this second cane the transmission chamber contained potted shoots of eyes 1x, 3x, 5x, and 13x. Cuttings 7x, 9x, and 11x were discarded at germination because the shoots were broken. The control chamber contained potted shoots from eyes 2x (dead), 4x, 6x (dead), 8x, 10x, 12x, and 14x.

On August 5, 1932, shoots 1x and 13x had very young Fiji galls on the nether surface of the leaves and on the midribs, an incubation period of 49 days. Twenty-four days later, shoot 3x showed the disease, an incubation period of 73 days. The first symptom of Fiji disease infection of shoot 5x was noted on September 1, an incubation period of 76 days. All check shoots were vigorous and free from Fiji disease.

The development of Fiji symptoms on the leaves and midribs of 100 per cent of the odd-numbered one-node cuttings of POJ 2878 sugar cane used in the two experiments described above is attributed to transmission by the adult *P. vastatrix* employed in the experiments. That infection by the Fiji disease could not be accidental is shown by the fact that all of the even-numbered cuttings used as checks remained healthy and free from Fiji galls.

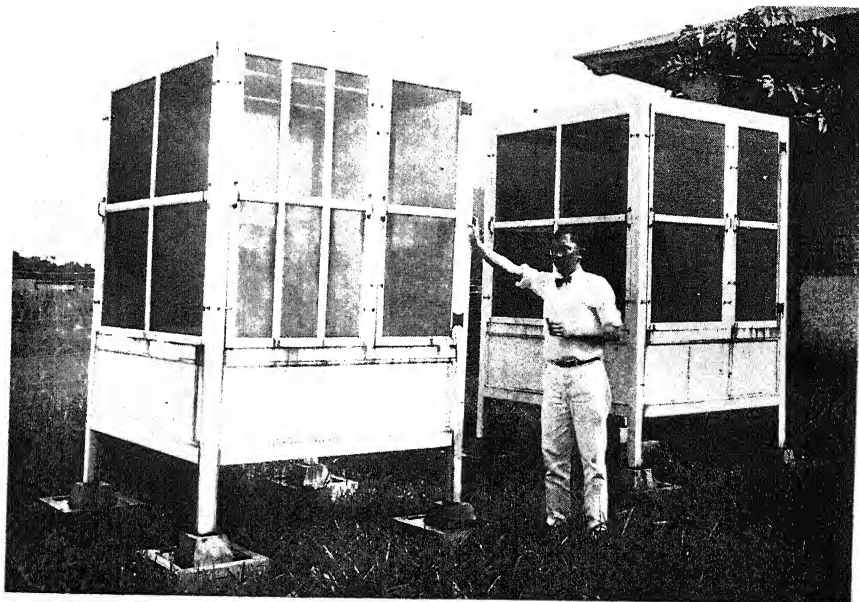
Experiment 3. In this as in the preceding experiments one-node cuttings were used from a single cane of POJ 2878, the odd numbers being given to nodes for the transmission trials, the even numbers for checks. On September 5, 1932, the outer surface of the furled youngest leaf of each of the potted cuttings 1*a*, 3*a*, and 5*a* was marked with India ink. These shoots were about 13 days old and had been covered with cheesecloth since they had emerged through the soil. Fifteen adult leaf-hoppers, *P. vastatrix*, were then introduced under the cheesecloth covers of shoots 3*a* and 5*a*. These leaf-hoppers came from the Fiji-infected canes of experiments 1 and 2 in the insect-proof chamber. At this same time leaves and leaf sheaths from the infected canes of experiments 1 and 2 which contained eggs and newly hatched leaf-hoppers were placed inside the cloth cover of shoot 1*a*. On September 6, twenty adult leaf-hoppers from the same source were added to those on 5*a* and ten adult individuals to those on 3*a*. Ten young leaf-hoppers were also added to those on 1*a*.

On September 14, potted shoots 1*a*, 3*a*, and 5*a* were placed in the experimental insect-proof chamber and the cheesecloth covers were removed. The insects were not destroyed but were allowed to continue feeding. Potted shoots 2*a* and 4*a*, the checks, were placed in the control insect-proof chamber. Their cheesecloth covers were also removed.

On October 3, 1932, after an incubation period of 28 days, the youngest leaf of shoot 5*a* had very young galls on the nether surface of the upper end of the leaf near its margin. This was the fourth leaf produced after the introduction of the 35 adult leaf-hoppers.

On October 24, 1932, the first young gall on shoot 3*a* appeared on the nether surface of the upper portion of the leaf, an incubation period of 40 days. This leaf was the fifth to be produced after the introduction of 25 adult leaf-hoppers on this shoot.

On December 9, 1932, the first symptom of the Fiji disease appeared on the youngest leaf of shoot 1*a*. Calculating from the day that shoots 1*a*, 3*a*, and 5*a* were placed in the insect-proof transmission chamber, the incubation period of the Fiji disease in shoot 1*a* was 86 days. This long incubation period in shoot 1*a* may seem attributable to absence of infective material in the young of leaf-hoppers which were taken from infected canes and in those hatched from the eggs laid on infected shoots. Shoot 1*a* was exposed to infective adult leaf-hoppers only when it was placed with shoots 3*a* and 5*a* in the insect-transmission chamber on September 14, 1932. Shoot 1*a* came from the oldest node, and it was the slowest in growth of all the shoots.



OCFEMIA: SUGAR CANE

shorter is the incubation period of the disease. The check shoots 2a and 4a were free from Fiji symptoms and grew vigorously, thus confirming the conclusions following experiments 1 and 2.

Experiment 4. On October 4, 1932, potted shoots 1b and 3b from one stalk of POJ 2878 and potted shoots 1c, 3c, 5c, 7c, and 9c of another stalk were placed in the insect-transmission chamber. Potted shoots 2b and 4b of the first stalk and 2c, 4c, 6c, and 8c of the second stalk were placed in the check chamber. Adults of *P. vastatrix* which had been confined on infected shoot 13x from experiment 2 were introduced into the transmission chamber. At the end of 92 days 100 per cent of the odd-numbered shoots of the two stalks were diseased, while the checks were clean and healthy.

To determine if adults of *P. vastatrix* that came from healthy cane can cause Fiji disease, shoots 5b, 6b, 7b, and 8b of a stalk of POJ 2878 sugar cane were covered with cheesecloth. On September 9, 1932, 26 adults of *P. vastatrix* were allowed to feed on shoot 6b and 50 adult leaf-hoppers on shoot 8b. Shoots 5b and 7b (odd-numbered one-node cuttings) without leaf-hoppers were checks. On September 21, 1932, more adult Fiji-free leaf-hoppers were added on shoots 6b and 8b. On November 10, 1932, the odd-numbered shoots were placed in the transmission chamber where there were leaf-hoppers from diseased canes, and the even-numbered shoots with the Fiji-free leaf-hoppers in the check chamber, and their covers were removed. Up to February 3, 1933, shoots 6b and 8b were free from Fiji disease, while shoots 5b and 7b were diseased. This insect, however, caused much injury to the cane shoots 6b and 8b by sucking the juice of the plants and depositing eggs in the punctures on the midribs and leaf sheaths.

THE FIRST SYMPTOMS PRODUCED IN INSECT-TRANSMISSION EXPERIMENTS

In Fiji transmission experiments with the use of leaf-hoppers the first symptom of the disease that appeared was the presence of galls on any por-

EXPLANATION OF PLATE 1

Fig. 1 (top). The insect-proof cages used in experiments on Fiji transmission. The one at the left was used as the "transmission chamber." In this were grown the experimental canes given odd numbers, along with the leaf-hoppers from Fiji-infected canes. In the one at the right grew the control canes (even-numbered), free from insects.

Fig. 2 (center). The experimental (odd-numbered) and check (even-numbered) shoots from one-node cuttings of one stalk of POJ 2878 sugar cane used in experiment 1. Shoot from node 3 did not germinate, and shoot from node 7 was discarded before the experiment was started. All the new leaves of the experimental shoots developed galls, while the leaves of the checks were clean and free from galls. Note that shoots 1, 13, and 15 are beginning to produce shorter leaves than the checks. Photographed September 6, 1932.

Fig. 3 (bottom). Photograph of experiment 1 taken November 23, 1932, 78 days after the photograph of figure 2 of this plate was taken. The photograph shows the contrast between the infected and the healthy shoots. Note the size and appearance of shoots 1, 5, 9, 11, 13, and 15 as compared with shoots 2, 4, 6, 8, 10, 12, 14, and 16. Shoot 11 has a long stalk which was not cut on May 4, 1932. This illustrates infection after

tion of the nether surface of the lamina of the leaf (fig. 2) or on the nether surface of the midrib. The galls were present either near the base of the leaf, in the middle, or near the tip (fig. 2). They varied in size from one and one-half to two millimeters in length by one-third to one-half millimeter in diameter. A string of these small galls was formed, and the chain ranged from five to ten centimeters in length. Shoot 7c in experiment 4 had a gall 38 mm. long and 2 mm. wide on the midrib at the base of the youngest expanded leaf, 15 days after the appearance of the first symptoms. In the very early stage of the disease the galls could hardly be distinguished from the rest of the leaf surface because their color was much like the rest of the leaf. By feeling with the finger, however, the galls could be easily detected. In a few days the galls became straw-colored. After from two to three weeks some of the galls began to break open and turned brown.

Another effect of the disease is illustrated in plate 1. It is that, under these carefully controlled conditions, a shortening of the subsequent leaves took place two to three weeks after the appearance of the first symptoms of infection. The appearance of the experimental shoots as compared with the check shoots, 110 days after the first symptoms were noted, is shown in figure 3 of plate 1. No changes were noted in the roots of the infected plants.

The youngest leaf of shoots 3a and 5a rotted 63 days after the appearance of the first symptoms.

SUMMARY

The results of experiments under carefully controlled conditions in insect-proof chambers, using one-node cuttings of POJ 2878 sugar cane, show that the Fiji disease can be transmitted by adults of the leaf-hopper *Perkinsiella vastatrix* Breddin. The incubation period of the disease in sugar cane varied from 28 to 86 days.

Adults of the leaf-hopper from healthy canes did not cause Fiji disease.

The first symptom of the disease in these transmission experiments was the presence of very small galls on any part of the nether surface of the leaves and midribs.

Shortening of the leaves occurred two to three weeks after the appearance of the first symptoms.

The experimental work on insect transmission of the Fiji disease is being continued by the writer at Los Baños, including further trials with this leaf-hopper and other species of insects.

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ROOT GRAFTING IN TREES¹

CARL D. LA RUE

(Received for publication February 10, 1933)

Rigg and Harrar (1931) in a study of root systems growing in sphagnum report that "excessive root fusion often occurs in sphagnum peat." They attribute the fusion to the swaying of the trees, which wears away the bark on the roots so that the meristematic areas of different roots are brought into contact. They state also that fusions of the stems of *Alnus oregana* which have been rubbed together by swaying in the wind are common in the Seattle region.

Small (1932) has described natural grafts in stems of four species of trees. He believes that rubbing together of the stems, or branches, is the cause of the fusions.

Millner (1932) has studied stem grafting in *Hedera helix* and has shown that attrition of the bark by rubbing of stems, or by other means, is unnecessary in the process.

Examples of stem grafting in forest trees are known to most botanists and are generally thought to be due to wearing away of the bark at the points of contact by the waving of the branches in the wind. However, the writer has seen grafts in tree stems which could not be explained in this way, and in tropical countries he has observed, in various species of strangling figs, thousands of grafts where the bark had not been disturbed in any way by external means. The strangling figs represent the most remarkable series of natural grafts to be seen anywhere, and it appears that the lightest contact between two branches or two roots is sufficient to cause their union. From these observations the author has been led to question whether any removal of bark by friction is needed to bring about grafting in tree roots.

Comparatively little is known about the root systems of our forest trees, and it is a matter of regret that earlier botanists did not undertake a study of tree roots at a time when great areas in the United States were being denuded of their forests. So little land is being cleared at the present time that one seldom has an opportunity to observe many root systems of any one species. The cost of grubbing out mature trees for the purpose of making studies on their roots is, of course, prohibitive.

Occasionally, opportunities to make root studies on one or two species are offered by circumstances, and it is to be hoped that botanists will take advantage of them before they become even fewer. The present paper represents the utilization of such opportunities.

¹ Papers from the Department of Botany, University of Michigan, No. 405.

SPECIES IN WHICH ROOT GRAFTS ARE COMMON

Pinus strobus. In these days few of us ever see the root system of a mature white pine exposed, but in the preceding generation vast numbers of stumps of this species were grubbed out, as the areas left by the lumberman were turned into fields. The greater number of the stumps were burned as they were removed, but in certain sections of the state of Michigan and elsewhere great numbers of them were drawn into lines along the boundaries of fields to serve as fences. Here they have remained, undergoing a very gradual decay.

In the neighborhood of Alma, Michigan, there are still hundreds of miles of these fences of white-pine stumps. They are still sound enough to burn well, and many of them are being cut up as a cheap source of fuel.

In this region the writer drove along roads bordered by stump fences for at least ten miles, and according to a conservative estimate he must have seen 3000 stumps in sufficient detail to recognize root grafts on all of them. Not a single stump was seen which did not show some grafts among its roots.

Most of the small roots were cut away when the fences were made, and the remainder have decayed so that only the larger roots are left to show what the original conditions may have been. But as the stumps now appear, the roots are grafted wherever they come into contact with one another. Originally the number of grafts must have been much greater than one can now find, because many of the small roots still present show unions and the roots which have been lost must have borne a great many more.

Even as they now lie, the stumps frequently are partially buried in the soil and débris, so that numerous grafts are hidden, and there are complexes of roots which conceal many others. Table I presents the data secured from actual counts on 50 stumps, and figure 1 shows the actual appearance of a typical example. In almost every case there were undoubtedly other junctions which were partially or completely concealed.

TABLE I. *Root grafts on stumps of Pinus strobus and Thuja occidentalis*

Numbers of root grafts	Stumps of <i>Pinus strobus</i>	Stumps of <i>Thuja occidentalis</i>
1-10		22
11-20		31
21-30	4	19
31-40	11	9
41-50	12	4
51-60	8	7
61-70	5	3
71-80	3	5
81-90	1	—
91-100	3	—
101-110	1	—
111-120	2	1
Totals	50	101

In only one instance were the roots of two stumps found grafted together. Here a small stump was united to a large one by unions of several large roots, so that the two could have been separated only with difficulty (fig. 1). Such unions of separate root systems could not have been very numerous because the large trees did not form a very close stand, but several men who have

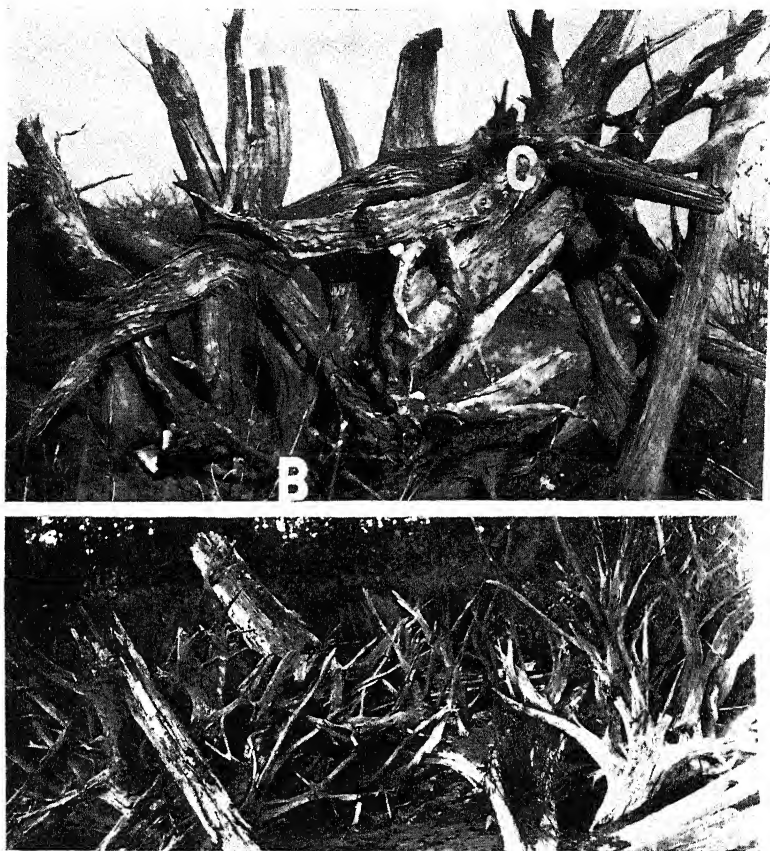


Fig. 1 (above). Root grafts in *Pinus strobus*. Two separate trees, whose roots are fused together, are represented. The base of a large tree is shown at *B*; that of a smaller one at *C*.

Fig. 2 (below). Grafts on the roots of *Thuja occidentalis*. This plexus involves the roots of several trees.

built stump fences in the past have assured the writer that stumps were often so firmly united that they had to be pulled at the same time and chopped apart before they could be placed in the fence rows.

Although definite counts were not made to settle the question, it appeared that the root unions were more numerous on stumps which had grown on

low land. The roots were smaller in the lower lands, and it may well be that they were confined to the upper layers of the soil by the high-water table. The greater competition of the roots in the upper soil would be expected to give rise to more root contacts and more grafts. Stumps of trees grown on sandy upland soil and on clay hills showed larger roots, but there was no lack of grafts on any of these.

Thuja occidentalis. Along a small stream, known locally as Nigger Creek, which flows into Mullet Lake near Topinabee, Michigan, the writer found a number of unprotected trees of *Thuja occidentalis*. Apparently the meandering stream has cut away the soil from these trees so that they have toppled over with their roots exposed. These trees are rather small, ranging from 10 to 100 cm. in diameter, and nearly all the roots are small. The roots were confined to a layer scarcely more than 30 cm. thick, and competition between them was obviously severe.

The soil here is a black muck, easily penetrated by the roots, but sufficiently firm to give good support to the trees. All but the smallest roots are still intact on most of the trees, so that one can see that they were exceedingly numerous and that contacts between them were very common. Whenever they came into close contact they have grown together. Table 1 shows the numbers of grafts counted on 101 of these trees, and figure 2 gives an idea of their appearance. It is certain that only a small part of the grafts was visible, for compact masses of small roots were often encountered in which great numbers of unions were concealed.

Grafts between roots of different trees were very common, since the trees had grown in a close stand. Of the trees listed in table 1, 64 showed unions with at least one other tree. In one place 11 stumps were completely knitted together, so that it was often impossible to tell to what tree a root emerging from the plexus originally belonged.

Ulmus americana. Only a small number of trees of this species has been available for study. None of these has been without grafts, and most of them have shown numerous unions regardless of the type of soil in which they grew.

Tilia americana. Observations on limited numbers of trees of this species reveal root unions in abundance. In one tree that had grown in low ground the roots were spread in a thin flat plate not over 10 cm. thick. The tree had been blown over so that this thin root mass was fully exposed. The roots were seen to be united almost into a solid mass by myriad grafts. It seems that this species is one in which root fusions occur very readily—more readily than in most others.

Betula lutea. Grafts are very numerous on roots of yellow birch trees. The trees examined were growing on a black soil in a rather wet location, and accordingly the roots were spread out in a thin layer near the surface, where they crossed one another repeatedly and fused at nearly every crossing.

Acer saccharinum. Under conditions identical with those described for *Betula lutea*, trees of *Acer saccharinum* produce great numbers of root grafts.

SPECIES WHICH DO NOT FORM ROOT GRAFTS READILY

Larix laricina. A few larch trees were seen which had been uprooted so that all but the outer ends of the roots could be seen. It was obvious that if any root grafts were formed they were very few. Without a complete dissection of the root systems it was impossible to be certain that no grafts were present, but none could be found on the smaller roots. Many roots were found which lay in grooves in others, but in none of these was there any fusion whatsoever. This behavior contrasted strikingly with that of the roots of all the species described earlier in this paper.

The tamarack trees had grown in the lowest and least solid ground of any observed in this study, so that they should have had greater opportunity to sway and wear away the bark between the roots than any of the others.

Populus tremuloides. The roots of this species appear to fall into the same class as those of the tamarack. No root grafts could be made out with certainty. None were found between any of the roots which could be flexed, for several apparent fusions proved not to be what they seemed. The roots could always be pulled apart, showing that they had grown around one another, but had not fused at all. It is possible that in some of the large roots which are forced together by continued growth actual fusions may occur, but none has been seen.

Fraxinus nigra. Root fusions do not appear to take place between roots of the black ash, but too few trees have been examined to determine the facts for this species.

Prunus serotina. The exposed roots of a few trees have been seen, and on these no grafts have been found. One tree was found in a wet habitat, and in this the root crown was set up about 20 cm. above the soil, with small roots extending down into the earth. This was a small tree and could be swayed back and forth with the hands, so that the roots were flexed and rubbed against one another. It appeared that the winds had swayed this tree, for some of the roots showed abraded and worn surfaces where they came into contact, but the cambium had not been exposed sufficiently to induce any fusions.

GRAFTS BETWEEN DIFFERENT SPECIES

Roots of *Betula lutea* were found which were firmly united to roots of *Ulmus americana*. There could be no question that a strong union was formed, for the roots could not be pulled apart. The exact nature of the fusion remains to be determined.

Some roots of *Betula lutea* appeared to be grafted with those of *Acer saccharinum*, but since the roots were large, they could not be bent so as to determine whether the roots were merely grown around each other or really fused.

DISCUSSION

Millner (1932) has shown that stems of *Hedera helix* grow together without the exposure of the cambium by any external means. Small (1932) mentions the rubbing of branches as a possible factor in stem and branch grafts in four species, but shows a picture of a *head-on* graft between branches of *Taxodium ascendans* in which abrasion must have played a minor rôle.

Rigg and Harrar (1931) believe that root fusions in *Pinus monticola* growing in sphagnum are due to attrition of the bark as the roots are flexed when the trees are blown back and forth by the wind. Root grafting in *Pinus strobus* cannot be caused by wind action because large trees growing in stiff clay could not conceivably have been swayed enough to wear away the bark on the roots before the fusions took place. It is hardly more likely that the vast number of root unions in *Thuja americana* could have a connection with wind action than that those of *Pinus strobus* could.

Tamaracks growing in peaty soil where wind sway might be possible do not bear root grafts, and the one tree of cherry which showed some abrasion of bark, apparently due to wind action, produced no root fusions.

The soil plays a part in the grafting of roots in *Pinus strobus*, *Thuja occidentalis*, *Ulmus americana*, *Betula lutea*, *Tilia americana*, and *Acer saccharinum*, by serving as a support that prevents growth in diameter from pushing apart the roots which have come into contact with each other. On the contrary, it tends to keep the roots pressed firmly together so that the reactions which result in fusions may be induced. The nature of the root systems of a given species may be such as to give rise to a great or to a small number of root contacts. The nature of the cambium probably determines whether or not root grafts take place readily between roots held together by the combined action of centrifugal growth and the soil.

SUMMARY

1. Root grafts are common in *Pinus strobus*, *Thuja occidentalis*, *Ulmus americana*, *Betula lutea*, *Tilia americana*, and *Acer saccharinum*.
2. The roots of *Larix laricina*, *Fraxinus nigra*, and *Prunus serotina* seem not to fuse together readily.
3. Removal by friction of the bark at the point of contact between two roots is not necessary to produce root grafts in the species discussed in this paper.

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NEW SPECIES, AND CHANGES IN NOMENCLATURE, OF GRASSES OF THE UNITED STATES

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During the preparation of a Manual of the Grasses of the United States several new species have been found which it seems advisable to publish in advance of the manual.

The original descriptions of all the grasses from the United States, scattered through a vast quantity of literature, have been examined, with the result that several well-known names must be replaced by earlier ones. The systematic sequence of the notes is that given in the Genera of Grasses of the United States.¹

ARUNDINARIA GIGANTEA (Walt.) Chapm. Fl. South. U. S. 561. 1860.

Arundo gigantea Walt. Fl. Carol. 81. 1788.

Arundinaria macrosperma Michx. Fl. Bor. Amer. 1: 74. 1803.

Walter (*op. cit.*) describes two species of *Arundo*, *A. gigantea* and *A. tecta*. There is a fragmentary specimen of the first species in the Walter Herbarium at the British Museum of Natural History. The second species is not represented. Chapman uses the specific name *gigantea* without reference to Walter, but gives *Arundinaria macrosperma* Michx. as a synonym. Since he rejects *macrosperma* and takes up *gigantea*, we may assume that he is basing his name on *Arundo gigantea* Walt. *Arundinaria gigantea* Nutt.² is published only as a synonym of *Meigia gigantea* Nutt., which is based (through Elliott) on *Arundo gigantea* Walt.

BROMUS CATHARTICUS Vahl, Synb. Bot. 2: 22. 1791.

Bromus unioloides H. B. K. Nov. Gen. & Sp. 1: 151. 1816.

The type specimen of *B. catharticus*, collected at Lima, Peru, is in the Copenhagen Herbarium. Through the courtesy of Dr. Christensen, the curator, I have been able to examine a fragment of this.

BROMUS BREVIARISTATUS Buckl. Proc. Acad. Phila. 1862: 98. 1863.

Bromus subvelutinus Shear, U. S. Dept. Agr. Div. Agrost. Bull. 23: 52. 1900.

The type of Buckley's species has been examined in the Herbarium of the Academy of Natural Sciences, Philadelphia. It was collected in the Rocky Mountains by Nuttall. This species has been referred to *B. marginatus* Nees, but the narrow canescent blades show that it is what Shear described as *B. subvelutinus*. Buckley's original description states, "Whole plant pilose, with short erect hairs." Buckley cites "*Ceratochloa breviaristata*? Hook." as a possible synonym, but the name is not based on that, Nuttall's plant being described and cited.

¹ U. S. Dept. Agr. Bull. 772. 1920.

² Gen. Pl. 1: 39. 1818.

Bromus anomalus Rupr.; Fourn. Mex. Pl. 1: 126. 1886.

Bromus kalmii var. *porteri* Coulter, Man. Rocky Mount. 425. 1885.

Bromus porteri Nash, Bull. Torrey Club 22: 512. 1895.

Fournier cites several specimens, among them *Galeotti* 5757, earlier listed by Ruprecht without description. This specimen and two other specimens cited by Fournier were examined in the herbarium of the Museum d'Histoire Naturelle, Paris.

Bromus mollis L. Sp. Pl. ed. 2. 1: 112. 1762.

Holmberg³ differentiates *Bromus hordeaceus* L. and *B. mollis* L., the former being a rare species of northwestern Europe with smaller spikelets, the lemmas mostly glabrous, whereas the latter is the common widespread species with villous spikelets, which is introduced in the United States and hitherto known as *B. hordeaceus*. None of our specimens agrees with true *B. hordeaceus*.⁴

Festuca dertonensis (All.) Aschers. & Graebn. Syn. Mitteleur. Fl. 2: 588. 1900.

Bromus dertonensis All. Fl. Pedem. 2: 249. 1785.

This species has been known in the United States as *F. bromoides* L. That name appears to be based on a mixture and is rejected by European authors as uncertain.

Festuca elmeri var. *conferta* (Hack.) Hitchc.

Festuca jonesii var. *conferta* Hack.; Beal, Grasses N. Amer. 2: 593. 1896.

Festuca kingii Cassidy, Colo. Agr. Exp. Sta. Bull. 12: 36. 1890.

Festuca confinis Vasey, Bull. Torrey Club 11: 126. 1884.

Festuca kingii, possibly based on *Poa kingii* S. Wats., though that is not cited, has been rejected because of *Poa kingiana* Steud. 1854. Both names are valid under the International Rules.

Festuca kingii var. *rabiosa* (Piper) Hitchc.

Festuca confinis rabiosa Piper, Contr. U. S. Nat. Herb. 10: 41. 1906.

Glyceria canadensis var. *laxa* (Scribn.) Hitchc.

Panicularia laxa Scribn. Bull. Torrey Club 21: 37. 1894.

Glyceria laxa Scribn.; Rand & Redfield, Fl. Mt. Desert 180. 1894.

Glyceria otisii Hitchc., sp. nov.

Perennis; culmi erecti, 100–125 cm. alti; laminae planae, scabrae, 10–15 cm. longae, 8–12 cm. latae; panicula 15–20 cm. longa, ramis patulis, gracilibus; spiculae oblongae, 5–7 mm. longae, 5–7-florae; glumae inaequales, secunda obovata, 1.2 mm. longa et lata; lemmata oblonga, 7-nervia, nervis scabris, 2.5–3 mm. longa, infra apicem purpurea, apice ipso valde hyalino-limbata.

Perennial; culms erect, 4–6-noded, glabrous, 100–125 cm. tall; sheaths glabrous, shorter than the internodes; ligule thin, 2–3 mm. long; blades flat, linear-lanceolate, scabrous on both surfaces and margin, somewhat spreading, 10–15 cm. long, 8–12 mm. wide; panicle open, 15–20 cm. long, nodding, the axis glabrous below, scabrous above, the slender branches scabrous, spreading or drooping, the lowermost in twos to fours, the others mostly in pairs, the

³ Bot. Not. 1924: 325. 1924.

⁴ See also Pilger, Verh. Bot. Ver. Brand. 74: 94. 1932.

lower internodes 2-3 cm. long, all naked below, bearing a few appressed branchlets, these with 1-3 spikelets on pedicels 2-4 mm. long; spikelets oblong, 5-7 mm. long, 5-7-flowered, the internodes of rachilla glabrous, about 0.5 mm. long; glumes unequal, rounded on the back, glabrous, thin, nerveless, except the rather faint keel, very finely ciliate-toothed at summit, the first ovate, acutish, pale, a little more than 1 mm. long; the second obovate, rounded or almost truncate at apex, about 1.4 mm. long and about as wide above the middle; lemmas overlapping about one-half, oblong, slightly compressed, strongly 7-nerved with sometimes an additional short pair of nerves, rather strongly scabrous on the nerves and here and there on the internerves and especially near the margin, 2.5-3 mm. long, the summit rounded or acutish, scarcely narrowed toward the tip, a prominent hyaline border contrasting with a dark purple zone just below, the border minutely erose-dentate and very finely ciliate like the glumes; palea broad, thin, nearly as long as the lemma, minutely notched at tip, the keels ciliate-scabrous; anthers about 0.8 mm. long.

Type in U. S. National Herbarium, no. 1538644, collected in a small creek near Mile 15 on trail to Hoh, Jefferson County, Washington, alt. 100 meters, July 10, 1927, by I. C. Otis (no. 1548).

The species is allied to *Glyceria elata* (Nash) Hitchc., but differs in the broader oblong spikelets, with, on the average, more florets, the broader glumes and lemmas, especially at the summit, the very scabrous lemmas, and the prominent hyaline minutely ciliate erose-dentate tip contrasting with the purple zone just below, the lower part of the lemma being green. No other collection has been seen. The specimens were sent to me by Mr. J. W. Thompson, who stated that Professor St. John had suggested to him that they represented an undescribed species.

GLYCERIA NEOGAEA Steud. Syn. Pl. Glum. 1: 285. 1854.

Glyceria pallida var. *fernaldii* Hitchc. Rhodora 8: 212. 1906.

Glyceria fernaldii St. John, Rhodora 19: 76. 1917.

Puccinellia pumila (Vasey) Hitchc.

Glyceria pumila Vasey, Bull. Torrey Club 15: 48. 1888.

Glyceria paupercula Holm, Repert. Sp. Nov. Fedde 3: 337. 1907.

Puccinellia paupercula Fern. & Weath. Rhodora 18: 18. 1916.

POA LAXIFLORA Buckl. Proc. Acad. Phila. 1862: 96. 1863.

Poa leptocoma elatior Scribn. & Merr. Contr. U. S. Nat. Herb. 13: 71. 1910.

Poa remissa Hitchc. Proc. Biol. Soc. Washington 41: 158. 1928.

An examination of the fragmentary type of *P. laxiflora* at the Academy of Natural Sciences, Philadelphia, shows that it is the same as *P. remissa*. It had been previously referred to *P. leptocoma* Trin.

ERAGROSTIS PECTINACEA (Michx.) Nees, Fl. Afr. Austr. 406. 1841.

Poa pectinacea Michx. Fl. Bor. Amer. 1: 69. 1803.

Poa caroliniana Spreng. Mant. Fl. Hal. 33. 1807.

Eragrostis purshii Schrad. Linnaea 12: 451. 1838.

This name has been in general use for *E. spectabilis* (Pursh) Steud. In the first edition of Gray's Manual (1848) *Poa pectinacea* Michx. is given as a synonym under *Eragrostis pilosa* (L.) Beauv., to which it is closely allied. In the second edition (1856) the synonymy of *Eragrostis purshii*, recognized

as distinct from *E. pilosa*, includes "*Poa caroliniana* Spreng., *P. pectinacea* of authors, not of Michx.," whereas the name *E. pectinacea* is applied to the perennial species (*E. spectabilis*) which has since generally been known under that name.

***Eragrostis tracyi* Hitchc., sp. nov.**

Perennis (?); culmi erecti, 30–80 cm. alti; vaginae glabrae, apice paullum pilosae; laminae planae vel involutae, glabrae, 5–25 cm. longae, 1–3 mm. latae; panícula erecta, patula, 10–15 cm. longa, 5–8 cm. lata, in axillis glabra, ramis tenuibus, ascendentibus vel patulis, flexuosis; spiculae lineares, plerumque 9–15-flores, 5–10 mm. longae, 1.5 mm. latae, paullum purpurascens, pedicellis flexuosis patulis 2–5 mm. longis; glumae acutae, 1 et 1.5 mm. longae; lemmata laxè imbricata, 1.5–2 mm. longa, nervis manifestis; palea subpersistens.

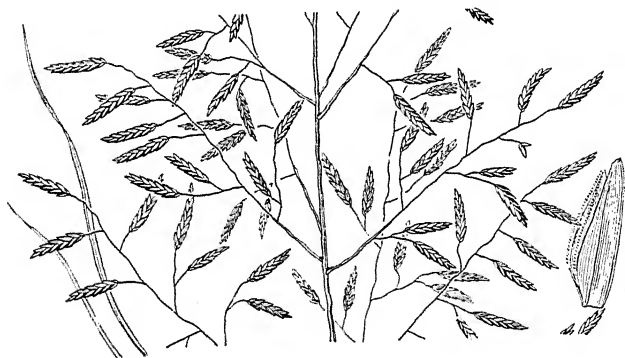


Fig. 1. *Eragrostis tracyi*. Natural size; floret $\times 10$. From type.

Apparently perennial; culms erect or somewhat spreading, branching at base, 30 to 80 cm. tall; sheaths glabrous, rather sparsely pilose at the throat; ligule a dense line of hairs less than 0.5 mm. long; blades flat or loosely involute, those of the innovations involute, glabrous, 5 to 25 cm. long, 1 to 3 mm. wide; panicle erect, open, 10 to 15 cm. long, 5 to 8 cm. wide, the axils glabrous or nearly so, the branches slender, ascending to spreading but not horizontal, flexuous; spikelets linear, mostly 9 to 15-flowered, 5 to 10 mm. long, about 1.5 mm. wide, pinkish or purplish, the pedicels flexuous, spreading, 2 to 5 mm. long; glumes acutish, the first about 1 mm. long, the second about 1.5 mm. long; lemmas 1.5 to 2 mm. long, the texture rather soft, rather loosely imbricate, the lateral nerves distinct; palea a little shorter than the lemma, minutely scabrous on the keels, somewhat persistent; caryopsis, about 0.7 mm. long. (Fig. 1.)

Type in the U. S. National Herbarium, no. 441983, collected on Sanibel Island, Florida, May 19, 1901, by S. M. Tracy (no. 7168). Also collected on Sanibel Island by A. S. Hitchcock in 1900.

ERAGROSTIS PILIFERA Scheele, *Linnaea* 22: 344. 1849.

Eragrostis grandiflora Smith & Bush, *Rep. Mo. Bot. Gard.* 6: 117. *pl.* 55. 1895.

Scheele cites New Braunfels, Texas, *Lindheimer*. A specimen of this

collection, which agrees with the original description, is in the U. S. National Herbarium.

UNIOLA SESSILIFLORA Poir. in Lam. Encycl. 8: 185. 1808.

Uniola longifolia Scribn. Bull. Torrey Club 21: 229. 1894.

Sprengel⁵ refers *Uniola sessiliflora* to *U. nitida* Baldw., but the description does not agree with that and does agree excellently with *U. longifolia*. Furthermore, *U. nitida* is not known from the Carolinas.

Neyraudia reynaudia (Kunth) Keng.

Arundo reynaudia Kunth, Rév. Gram. 2: 275. pl. 49. 1830.

TRIODIA GRANDIFLORA Vasey, Contr. U. S. Nat. Herb. 1: 591. 1890.

This species has been referred to *T. avenacea* H. B. K., a Mexican species, differing in having stolons and shorter purple panicles.

Orcuttia tenuis Hitchc., sp. nov.

Annual; culmi erecti, tenues, 5–15 cm. alti; folia plerumque basalia; ligula nulla; laminae involutae, 1–2 cm. longae; spica 3–6 cm. longa; spiculae sessiles, inferiores distantes, superiores approximatae, 3–10-flores, 10–15 mm. longae; glumae 3–4 mm. longae, 3-dentatae; lemmata 5 mm. longa, 15-nervata, 5-dentata, dentibus acuminatis setaceis; palea angusta lemma aequans, truncata.

Annual; culms erect, in small tufts, slender, sparsely and minutely pubescent below, glabrous above, 5 to 15 cm. tall, the nodes pubescent; leaves mostly basal, the sheaths glabrous, striate; ligule wanting; blades rather firm, involute, 1 to 2 cm. long, about 0.5 mm. wide when flat, pubescent on the upper surface, glabrous beneath or scabrous toward the tip; spike more than half the entire height of the plant, the lower spikelets distant, the upper approximate but not crowded; spikelets sessile or nearly so, purple-tinged, 3 to 10-flowered, 10 to 15 mm. long, the rachilla joints pubescent; glumes and lemmas strongly nerved, the glumes subequal, 3 to 4 mm. long, deeply and unequally 3-toothed, the teeth acuminate; lemmas firm, about 5 mm. long, 15-nerved, 5-toothed, the teeth awn-tipped, about equal, about one-third as long as the lemma, the rigid tips spreading or slightly recurved; palea narrow, obtuse or truncate, a little shorter than the lemma, deeply concave between the nerves.

Type in the U. S. National Herbarium, no. 734402, collected in open sandy soil, Goose Valley, Shasta County, California, June 29, 1912, by Alice Eastwood (no. 1013). This was distributed as *O. californica* (Amer. Gr. Nat. Herb. no. 686), and is the species illustrated in the Genera of Grasses of the United States.⁶

Agropyron subsecundum (Link) Hitchc.

Triticum subsecundum Link, Hort. Berol. 2: 190. 1833.

This is the species that has been going under the name of *A. caninum* L. Malte⁷ has shown that our species is distinct from the true European *A. caninum* which is distinguished by its 3-nerved glumes. Malte refers *Triticum subsecundum* to *Agropyron trachycaulum* var. *unilaterale* (Vasey) Malte. Although *A. subsecundum* and *A. trachycaulum* (*A. pauciflorum*) are closely

⁵ Syst. Veg. 1: 349. 1825.

⁶ U. S. Dept. Agr. Bull. 772: f. 38. 1920.

⁷ Nat. Mus. Canada, Ann. Rep. 1930: 27–48. 1932.

allied species there are relatively few intergrades and it appears to me better to recognize them as distinct.

***Agropyron subsecundum* var. *andinum* (Scribn. & Smith) Hitchc.**

Agropyron violaceum andinum Scribn. & Smith, U. S. Dept. Agr. Div. Agrost. Bull. 4: 30. 1897.

***Agropyron pauciflorum* (Schwein.) Hitchc.**

Triticum pauciflorum Schwein. in Keating, Narr. Exp. St. Peter's Riv. 2: 383. 1824.

Triticum trachycaulum Link, Hort. Berol. 2: 189. 1833.

Agropyrum tenerum Vasey, Bot. Gaz. 10: 258. 1885.

Agropyron trachycaulum Malte, Nat. Mus. Canada, Ann. Rep. 1930: 42. 1932.

Schweinitz cites "Prairies of the St. Peter" [Minnesota], collected by Thomas Say in 1823. The description leaves no doubt of the identity of the species, though the type was not found in the herbarium of the Academy of Natural Sciences, Philadelphia, where most of the Say collections are preserved. *Agropyron pauciflorum* (Schwein.) Hitchc. is not invalidated by *A. pauciflorum* Schur⁸ because that was not effectively published.

***Agropyron vulpinum* (Rydb.) Hitchc.**

Elymus vulpinus Rydb. Bull. Torrey Club 36: 540. 1909.

***Elymus hirtiflorus* Hitchc., sp. nov.**

Perennis, rhizomatibus tenuibus repentibus; culmi erecti, 40–90 cm. alti; laminae firmae, involutae, glabrae; spica erecta, 5–15 cm. longa; spiculae 4–6-flores; glumae angustae, firmae, aristatae; lemmata hirsuta, 8–9 mm. longa, aristis 5–10 mm. longis.

Perennial, with slender creeping rhizomes; culms erect, tufted, 40 to 90 cm. tall; sheaths glabrous; ligule membranaceous, scarcely 0.5 mm. long; blades firm, flat or usually involute, scabrous on the upper surface, glabrous beneath, rather stiff, ending in a sharp fine point, ascending or appressed, 5 to 20 cm. long, 1 to 4 mm. wide when flat; spike erect, 5 to 18 cm. long, the rachis villous, pubescent; spikelets usually 4 to 6-flowered; glumes firm, narrow, rather obscurely nerved, hirsute to sparsely hispidulous, tapering into an awn about as long as the body, the entire length 1 to 1.5 cm.; lemmas hirsute, sometimes sparingly so, the lower 8 to 9 mm. long, the awns 5 to 10 mm. long. (Fig. 2.)

Type specimen in the U. S. National Herbarium, no. 1019435, collected along edge of river at Green River, Wyoming, June 25, 1895, by C. L. Shear (no. 284). Other specimens, all from Wyoming, are: Green River, alt. 2000 meters, moist alkaline banks, common, *Williams* 2333; Bill's Ranch, alt. 1300 meters, river banks, common, *Williams* 2860.

In the type both glumes and lemmas are hirsute or somewhat villous. In the two specimens collected by *Williams* the lemmas are less densely hirsute and the glumes are sparsely so.

***Elymus triticoides* var. *simplex* (Scribn. & Williams) Hitchc.**

Elymus simplex Scribn. & Williams, U. S. Dept. Agr. Div. Agrost. Bull. 11: 57. pl. 17. 1898.

⁸ Verh. Siebenb. Ver. Naturw. 10: 77. 1859, as synonym of *A. caninum* Roem. & Schult.

Elymus ambiguus* var. *strigosus* (Rydb.) Hitchc.Elymus strigosus* Rydb. Bull. Torrey Club 32: 609. 1905.*Elymus villiflorus* Rydb. Bull. Torrey Club 32: 609. 1905.

The two forms described by Rydberg differ from *E. ambiguus* Vasey & Scribn. only in the pubescence on the lemmas. In *E. ambiguus* the lemmas are glabrous or scaberulous; in *E. strigosus* the lemmas are strigose-pubescent; in *E. villiflorus* they are villous-pubescent. The last two are known only from the type collections, both from Boulder, Colorado. It seems best to refer them both to *E. ambiguus* as a single variety.



Fig. 2. *Elymus hirtiflorus*. Natural size; spikelet $\times 5$. From type.

HYSTRIX Moench, Meth. Pl. 294. 1794.

Type species, *Elymus hystrix* L. (*Hystrix patula* Moench).

Asperella Humb. Mag. Bot. Roem. & Ust. 7: 5. 1790. Not *Asprella* Schreb. 1789.

Some authors use *Asperella* Humb. for *Hystrix*, regarding *Asprella* and *Asperella* as distinct words, *Asperella* not being invalidated by *Asprella*. I regard *Asperella* and *Asprella* as being different spellings of the same word.

Asprella Schreb. 1789 refers to *Leersia*.

Asperella Humb. 1790 refers to *Hystrix*.

Willdenow, 1809, uses *Asprella*, but refers to *Hystrix*. Pfeiffer⁹ under *Asperella* says, "see *Asprella*." Under *Asprella* he has two series of citations, one referring to *Leersia* and one referring to *Hystrix*. Bentham and Hooker¹⁰ use *Asprella* Willd. for *Hystrix* and give *Asprella* Schreb. as a synonym of *Leersia*, and do not refer to *Asperella* at all. Hackel uses *Asprella* Willd. for *Hystrix*. Dalla Torre and Harms use *Asperella* Humb. for *Hystrix* and refer *Asprella* Willd. to it as a synonym. In indexes various species are found under both *Asprella* and *Asperella*. Usage indicates that botanists have regarded the two names as being different spellings of the same word. Thus *Asprella* Schreb. is a synonym of *Leersia*, 1788, and *Asperella* Humb. (*Asprella* Willd.) is a later homonym and should be rejected.

Hordeum nodosum var. *boreale* (Scribn. & Smith) Hitchc.

Hordeum boreale Scribn. & Smith, U. S. Dept. Agr. Div. Agrost. Bull. 4: 24. 1897.

Trisetum orthochaetum Hitchc., sp. nov.

Perenne; culmi erecti, tenues, 110 cm. alti; vaginae glabrae; ligula 3-4 mm. longa; laminae planae, scabrae, 8-20 cm. longae, 3-7 mm. latae; panícula pallida, circa 18 cm. longa, ramis tenuibus ascendentibus, inferioribus fasciculatis infra nudis usque ad 8 cm. longis; spiculae 3-flores, 8-9 mm. longae, rachilla sericeo-pilosa; lemmata 5-6 mm. longa, callo piloso; arista recta, circa 5 mm. longa.

Perennial; culms solitary, erect, slender, about 3-noded, 110 cm. tall; sheaths glabrous; ligule thin, truncate, erose, 3 to 4 mm. long on the upper leaves, shorter on the lower; blades flat, rather thin, scabrous, 8 to 20 cm. long, 3 to 7 mm. wide; panicle long-exserted, rather lax, nodding, pale green, slightly tinged with purple, about 18 cm. long, the axis glabrous below, scabrous above, the branches scabrous, filiform, loosely ascending, in somewhat distant fascicles, naked below, as much as 8 cm. long; spikelets short-pedicel and somewhat appressed along the branches, usually 3-flowered, 8 to 9 mm. long excluding awns, the rachilla appressed-silky, continued beyond the third floret, sometimes bearing a much reduced fourth floret, glumes acuminate, scabrous on the keel, the first 6 mm. long, the second a little longer and wider; lemmas rounded on the back, obscurely 5-nerved, very minutely scabrous on the upper part, the callus short-pilose, the summit acute or subacute, slightly erose-toothed, the awn arising about 2 mm. below the summit, straight or nearly so, exceeding the lemma 3 to 4 mm., the first floret about 6 mm. long; the second slightly shorter, the third floret somewhat reduced; palea narrower and shorter than the lemma. (Fig. 3.)

Type in the U. S. National Herbarium, no. 1535753, collected in boggy meadow, Bitterroot Mountains, alt. 1200 meters, near Lolo Hot Springs, Missoula County, Montana, July 23, 1908, by Agnes Chase (no. 5129). Known only from the type specimen.

SPHENOPHOLIS PALLENS (Spreng.) Scribn. *Rhodora* 8: 145. 1906.

Aira pallens Spreng. Mant. Fl. Hal. 33. 1807.

Eatonia aristata Scribn. & Merr. U. S. Dept. Agr. Div. Agrost. Circ. 27: 7. 1900.

Sphenopholis aristata (Scribn. & Merr.) Heller, *Muhlenbergia* 6: 12. 1910.

The species going under the name of *Sphenopholis pallens* in recent litera-

⁹ Nomenclator Botanicus 1: 296. 1873.

¹⁰ Gen. Pl. 3: 1117, 1207. 1883.

ture should be called *S. intermedia* (Rydb.) Rydb. See *Bartonia* 14: 34. 1932.

AIRA L. Sp. Pl. 63. 1753.

There are 14 original species of *Aira*, but all except two have subsequently been removed to other genera. Of these two, *A. praecox* and *A. caryophylla*, the first may be selected as the standard species. *Aira caespitosa* and its allies were segregated under *Deschampsia*.



Fig. 3 (left). *Trisetum orthochaetum*. Natural size; floret $\times 5$. From type.

Fig. 4 (right). *Digitaria subcalva*. Natural size; 2 views of spikelet, and fertile floret $\times 10$. From type.

***Calamagrostis canadensis* var. *scabra* (Presl) Hitchc.**

Calamagrostis scabra Presl, Rel. Haenk. 1: 234. 1830.

This variety has been referred to *Calamagrostis langsдорffii* (Link) Trin. A fragment of the type of *Arundo langsдорffii* Link, sent by Dr. Pilger from the Berlin Herbarium, shows that it is not an American species. The rachilla is very minute or wanting, the spikelets are smaller than in *C. scabra*, the glumes are thinner, showing the nerves distinctly, and the blades are narrower.

Agrostis exarata var. **monolepis** (Torr.) Hitchc.

Polypogon monspeliensis var. *monolepis* Torr. U. S. Rep. Expl. Miss. Pacif. 5: 366. 1857.

Agrostis exarata var. *microphylla* (Steud.) Hitchc. Amer. Journ. Bot. 2: 303. 1915.

ALOPECURUS CAROLINIANUS Walt. Fl. Carol. 74. 1788.

Alopecurus ramosus Poir. in Lam. Encycl. 8: 776. 1808.

Alopecurus geniculatus var. *ramosus* St. John, Rhodora 19: 167. 1917.

There is a fragmentary specimen of this in the Walter Herbarium at the British Museum of Natural History. The spikelets have an exserted awn. In my account of the grasses of Walter's Flora¹¹ I stated there was no specimen in the Walter Herbarium. On a recent visit (1930) to the British Museum I reexamined the collection and found the specimen mentioned above.

Muhlenbergia torreyana (Schult.) Hitchc.

Agrostis compressa Torr. Cat. Pl. N. Y. 91. 1819. Not *A. compressa* Willd. 1790, nor Poir. 1810.

Agrostis torreyana Schult. Mant. 2: 203. 1824.

Sporobolus compressus (Torr.) Kunth, Enum. Pl. 1: 217. 1833.

Sporobolus torreyanus (Schult.) Nash in Britton, Man. 107. 1901.

It is necessary to take up this specific name in spite of *M. torreyi* (Kunth) Hitchc.; Bush, 1919.

Muhlenbergia longiligula Hitchc.

Epicampes ligulata Scribn.; Vasey, Contr. U. S. Nat. Herb. 3: 58. 1892.

Not *Muhlenbergia ligulata* (Fourn.) Scribn. & Merr. 1901.

Epicampes Presl is characterized by the author as having a spikelike panicle, subequal convex obtuse glumes, a little shorter than the floret, and the midnerve of the lemma extending into a straight awn. The only species described is *E. strictus* but the author states that *Agrostis pubescens* H. B. K. and *A. lanata* H. B. K. belong to the genus. The last two species have glumes as long as the floret. *Epicampes macroura* (H. B. K.) Benth. and *E. rigens* (Thurb.) Benth. are closely allied to *M. acuminata* Vasey and *M. angustata* (Presl) Kunth. *Muhlenbergia emersleyi* Vasey and its allies show a transition to *Epicampes*. Presl himself described one of these allies as *Podosaemum distichophyllum*. The character which I have used to differentiate the two, the awn from the back of the lemma just below the apex in *Epicampes* and from the tip in *Muhlenbergia*, has several exceptions in the latter genus. Since it is impossible to find a clear line of division to separate the genera, I am uniting *Epicampes* with *Muhlenbergia*.

SPOROBOLUS POIRETII Roem. & Schult. Hitchc. Bartonia 14: 32. 1932.

Axonopus poiretii Roem. & Schult. Syst. Veg. 2: 318. 1817.

Sporobolus berterianus (Trin.) Hitchc. & Chase, Contr. U. S. Nat. Herb. 18: 370. 1917.

Axonopus poiretii is based on "*Agrostis compressa* s. *Milium compressum* Poiret Enc. meth. Suppl. 1. p. 259. no. 78." No. 78 is on page 258, not 259. Poiret on page 258 describes "78. *Agrostis compressa*" giving himself as author, "N" [nob.]. The description, based on a plant collected in [South?] Carolina by Bosc, applies excellently to *Sporobolus berterianus*, but by some

¹¹ The Identification of Walter's Grasses. Rep. Mo. Bot. Gard. 16: 40. 1905.

mischance "*(miliun compressum)*" is cited as a synonym. Roemer & Schultes quote Poiret's description but were misled by the synonym erroneously cited. On page 259 Poiret described a second *Agrostis compressa*, no. 82, based on "*Miliun compressum*—Swartz, Prodr. 24, & Flor. Ind. occident. 1. pag. 183. . . . (Descript. ex Swartz)." The description applies to Swartz's species, *Axonopus compressus* (Swartz) Beauv.

ARISTIDA BARBATA Fourn. Mex. Pl. 2: 78. 1886.

Aristida havardii Vasey, Bull. Torrey Club 13: 27. 1886.

Henrard¹² has pointed out the identity of the two species. He takes the date of *A. barbata* as 1881, whereas I take 1886 as the date of publication.¹³ I have assumed, however, that *A. barbata* has priority.

ARISTIDA AFFINIS (Schult.) Kunth, Rév. Gram. 1: 61. 1829.

Chaetaria affinis Schult. Mant. 2: 210. 1824.

Aristida palustris Vasey, Descr. Cat. Grasses U. S. 35. 1885.

TRAGUS BERTERONIANUS Schult. Mant. 2: 205. 1824.

Mr. C. E. Hubbard of the Kew Herbarium has pointed out to me that the description of *Lappago aliena* Spreng. does not well apply to the species which has been called *Tragus aliena* (Spreng.) Schult. and *Nasia aliena* (Spreng.) Scribn. but, according to Stapf, applies to *Pseudechinolaena polystachya* (H. B. K.) Stapf. Our species should be called *T. berteronianus* Schult. The type of this is in the Berlin Herbarium (Krug and Urban Herbarium). It was collected in Santo Domingo by Bertero.

Leptochloa panicoides (Presl) Hitchc.

Megastachya panicoides Presl, Rel. Haenk. 1: 283. 1830.

Leptochloa floribunda Doell in Mart. Fl. Bras. 2^a: 89. 1878.

SPARTINA PECTINATA Bosc; Link, Jahrb. Gewächsk. 1³: 92. 1820.

Spartina michauxiana Hitchc. Contr. U. S. Nat. Herb. 12: 153. 1908.

Dr. Pilger kindly sent me a photograph of the type specimen in the Berlin Herbarium and also a fragment of the inflorescence. It is the narrow-panicked short-spiked northern Coastal Plain form of the widespread inland species. The description was not adequate for identification and the species was not known until recently from south of Virginia. While most of Bosc's collections were made in the Carolinas, Link gives only North America for the locality. Dr. Rydberg took up this name in his Flora of the Rocky Mountains.

SPARTINA LEIANTHA Benth. Bot. Voy. Sulph. 56. 1840 (Feb. or March).

Spartina foliosa Trin. Mém. Acad. St. Pétersb. VI. Sci. Nat. 4¹: 114. 1840 (later than June).

CHLORIS ANDROPOGONOIDES Fourn. Mex. Pl. 2: 143. 1886.

Chloris tenuispica Nash, Bull. Torrey Club 25: 436. 1898.

The type of *Chloris andropogonoides*, from San Luis Potosí, Mexico, (Viret 1462), was examined in the herbarium of the Muséum de l'Histoire Naturelle, Paris.

¹² Med. Rijks Herb. Leiden 54A: 224. 1927.

¹³ For discussion of date see Contr. U. S. Nat. Herb. 10: 49. 1910.

CHLORIS SUBDOLICHOSTACHYA C. Muell. Bot. Zeit. 19: 341. 1861.

Chloris brevispica Nash, Bull. Torrey Club 25: 438. 1898.

A specimen of the type collection of *Chloris subdolichostachya*, Texas, Drummond 372, is in the U. S. National Herbarium.

BOUTELOUA SIMPLEX Lag. Var. Cienc. 2^a: 141. 1805.

Chloris procumbens Durand, Chlor. Sp. 16. 1808.

Bouteloua prostrata Lag. Gen. & Sp. Nov. 5. 1816.

Bouteloua procumbens Griffiths, Contr. U. S. Nat. Herb. 14: 364. 1912.

HIEROCHLOE OCCIDENTALIS Buckl. Proc. Acad. Phila. 1862: 100. 1863.

Hierochloe macrophylla Thurb.; Boland. Trans. Agr. Soc. Calif. 1864-65:

132. 1866; S. Wats. Bot. Calif. 2: 265. 1880.

The type of *Hierochloe occidentalis* is in the herbarium of the Academy of Natural Sciences, Philadelphia. It was collected in the "Columbia woods" by Nuttall. Gray, in his notes on Buckley's plants,¹⁴ refers the species to *H. borealis* (the same as *H. odorata* (L.) Beauv.), but the Nuttall specimen is the large-leaved Pacific Coast species.

***Digitaria subcalva* Hitchc., sp. nov.**

Perennis, caespitosa; culmi tenues, ascendentes vel basi decumbentes; vaginae papilloso-pilosae; ligula 2 mm. longa; laminae planae, 3-15 cm. longae, 1-3 mm. latae; racemi 2-4, tenues, approximati, 5-12 cm. longi, rachi triangulari, basi nuda; spiculae anguste oblongo-lanceolatae, 2.5-2.8 mm. longae; gluma prima obsoleta; gluma secunda et lemma sterile lemma fertile aequantia, internerviis paullum appresso-pubescentibus.

Perennial, tufted; culms slender, ascending from a curved, often creeping rooting base, 40 to 100 cm. tall; sheaths papillose-pilose; ligule about 2 mm. long; blades flat, scabrous, the lower pilose, 3 to 15 cm. long, 1 to 3 mm. wide, the midnerve prominent; inflorescence long-exserted; racemes 2 to 4, slender, narrowly ascending, 5 to 12 cm. long, approximate but not digitate, the rachis slender, triangular, the margins very narrow, scabrous, mostly naked at base for 1 to 1.5 cm., or with distant abortive spikelets; spikelets rather distant, single or in pairs, the short pedicel about 0.5 mm. long, the long pedicel about 2 mm. long, narrowly oblong-lanceolate, 2.5 to 2.8 mm. long, acute; first glume obsolete or wanting; second glume and sterile lemma equal, the internerves from obscurely to distinctly appressed silky-pubescent, the glume 5-nerved, the lemma 7-nerved; fertile lemma pale or drab, as long as the glume and sterile lemma or the acute tip slightly exceeding it. (Fig. 4.)

Type in the U. S. National Herbarium no. 1537173 collected on low hammock land (Scranton fine sand), near a marsh, at Plant City, Florida, October 26, 1932, by C. P. Wright. Only known from the type locality. The plants grow in large spreading tufts, sometimes a meter or more in diameter.

PANICUM PURPURASCENS Raddi, Agrost. Bras. 47. 1823.

Panicum barbinode Trin. Mém. Acad. St. Pétersb. VI. Sci. Nat. 1: 256. 1834.

Raddi's name was not taken up in the revision of *Panicum*¹⁵ because of "*Panicum purpurascens* H. B. K." 1816, an error, that name never having been published, and *P. purpurascens* Opiz, 1822. The latter proves to be a nomen nudum. *Panicum muticum* Forsk. (1775) has been used for this grass, but this species, described from Egypt, is uncertain.

¹⁴ Proc. Acad. Phila. 1862: 337. 1863.

¹⁵ Contr. U. S. Nat. Herb. 15: 33. 1910.

ERIANTHUS ALOPECUROIDES (L.) Ell. Bot. S. C. & Ga. 1: 38. 1816.

Andropogon divaricatus L. Sp. Pl. 1045. 1753.

Andropogon alopecuroides L. Sp. Pl. 1045. 1753.

Erianthus divaricatus Hitchc. Contr. U. S. Nat. Herb. 12: 125. 1908.

The two names published by Linnaeus occur on the same page. *A. divaricatus* coming first. Under the International Rules *Erianthus alopecuroides* is the valid name because *A. alopecuroides* was first transferred to the accepted genus.

***Andropogon virginicus* var. *glaucopsis* (Ell.) Hitchc.**

Andropogon macrourus var. *glaucopsis* Ell. Bot. S. C. & Ga. 1: 150. 1816.

***Sorghum vulgare* var. *drummondii* (Nees) Hitchc.**

Andropogon drummondii Nees in Steud. Syn. Pl. Glum. 1: 393. 1854.

Holcus vulgare drummondii (Nees) Hitchc. Proc. Biol. Soc. Washington 29: 128. 1916.

HOLCUS L. Sp. Pl. 1047. 1753.

Of the seven original species only one, *H. lanatus*, is still retained in the genus. The historic type is *H. sorghum*¹⁶ L. and is the type species of the genus under the American Code. Under the revised International Rules *H. lanatus* is to be taken as the standard species in order to conserve the names *Holcus* and *Sorghum* in the way they have been most generally used during the last century.

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WASHINGTON, D. C.

¹⁶ See Hitchcock, U. S. Dept. Agr. Bull. 772: 266. 1920.

THE RELATIVE GROWTH RATES AND INTERDEPENDENCE OF TOPS AND ROOTS OF THE BIENNIAL WHITE SWEET CLOVER, *MELILOTUS ALBA* DESR.

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This paper relates some investigations of the growth habits of the biennial sweet clovers, particularly of the biennial white sweet clover, *Melilotus alba* Desr. The problems considered are: the relative growth of roots and tops during summer and fall of the first growing season; the effect of the removal of the tops in late August or early September and also in early October on the subsequent growth of roots and crowns; and the effect of the removal of the tops on the above dates on the winter-resistance of the roots and crowns.

These problems relate particularly to the growth habits characteristic of the biennial sweet clovers growing in latitudes where the life cycle is broken into two distinct seasonal growth periods by the severity of the winter. The importance of these problems has received most recognition in the corn belt of the United States, but they are no doubt involved in the handling of the biennial sweet clovers throughout most of the United States and Canada and probably in all parts of the world having temperate or cold climates.

A number of stations have given attention to some phases of one or more of the problems stated. The Ohio station, in a comprehensive series of experiments, included all of them but mainly in connection with a nurse crop. Most of the investigations of these problems have been quite limited in scope, and in most cases casual; nevertheless, they point to certain generalizations.

The only special account of the growth habits of the biennial sweet clovers is given by Willard (1925, 1927, 1930) at the Ohio station. On the plots at Columbus he found that the roots of the spring seedlings attained their maximum weight and maximum nitrogen content in the fall of the first season. The plants were found to be nearly all tops (80 to 90 per cent or more) in their early stages of growth; but mostly roots, sometimes as much as 80 per cent, at the end of the first season. The percentage of root weight gained rapidly on that of the tops from July to the end of the season. He reports the occurrence of a period of greatly increased root growth during the latter part of the first season. Under the influence of a nurse crop the period of greatly increased root growth began about the first of October and continued till cold weather, whereas in the absence of a nurse crop, it was found to be covered mostly by the month of September. One discrepancy, however, should be noted. Data from the Clermont County Farm, about 100 miles

south of Columbus, show that on plots seeded there in April and without a nurse crop, most of the period of increased root growth occurred after September.

Whiting and Richmond (1926) refer to a period of increased root growth near the end of the first season. Snider (1927) found that on the best Illinois corn lands and also in southern Illinois the roots attained their maximum volume the first season.

In regard to the effect of the removal of the tops on the subsequent growth and the winter resistance, Coe (1917) observed much variation. He noted that fall pasturing and clipping resulted in poor growth the second season in some cases but not in others. He, however, attributed the injurious effects to the loss of protection the tops afford and not to the disturbances in root development. In Tennessee (1924) mowing in the fall was followed by a high mortality and by a retarded growth the second season. Frazer (1924) observed that plants pastured closely in the fall have small roots and heave out badly. Willard (1927) found that mowing in September was very detrimental on the Ohio plots seeded with a nurse crop. On areas mowed in September, even as late as the 28th, the weight of the roots in some cases was less than 50 per cent of the weight of the roots on the unmowed areas. Mowing in September was found to result in much winter-killing, almost 100 per cent when done early in September, whereas mowing after October had very little effect on subsequent growth or winter resistance.

The above reports suggest the following: That the growth of the biennial sweet clovers during the first season follows a well-established course which probably prevails as generally as the biennial habit itself, and is characterized particularly by greatly increased root growth near the end of the season; that this development of roots during their special growth period depends on the presence of the tops; that the effects on the growth of the roots that follow the removal of the tops, as in mowing and pasturing, may be very detrimental or of little consequence, depending on whether the tops are removed just previous to the special growth period of the roots or after it; and that the disturbances in the growth of the roots following the removal of the tops are accompanied by a low winter-resistance. These suggested generalizations are so involved in cultural practices that their verification is warranted wherever the biennial sweet clovers occupy a place of importance among the crops.

GENERAL MORPHOLOGY

A biennial sweet clover plant, after it has passed the seedling stage, consists of three recognizable parts: a root system, a crown, and the aerial shoots. The aerial shoots are commonly called the tops. The root system consists of a prominent primary root, sometimes branched, and small secondary roots. The crown during the first season consists of the hypocotyl and the buds, designated as crown buds, borne on its apex. Beyond the seedling stage the

hypocotyl and the primary root constitute a continuous root-like structure in which the part contributed by each is usually not well defined.

The aerial shoots are of two kinds: the vegetative shoot of the first season and the flowering shoots of the second season. The flowering shoots of the second season are the crown buds which are present but display only little activity during the first season's growth. The vegetative shoot, which is the dominant aerial growth of the first year, is killed by freezing temperatures and thus disappears at the end of the first growing season. It is an annual shoot. The root system and the crown are the biennial structures of the biennial sweet clovers.

The vegetative shoot holds an important place in the life cycle of the plant. It provides the roots and crowns with food for their growth and for the storage upon which the flowering shoots of the second season are largely dependent. It is in this vital connection of the vegetative shoot with the life cycle of the plant that mowing and pasturing during the first season can have detrimental effects on the subsequent growth and vitality of roots and crowns.

The vegetative and flowering shoots are commonly described as appearing in succession. This is accounted for partly by the fact that the flowering shoots, the crown buds of the first season, remain inconspicuous until late in the first season.

In the vicinity of Ames the crown buds greatly increase their rate of growth in late August or early September and soon attain a noticeable size, often expanding several leaves before fall growth ceases. In reality both kinds of shoots, along with the other structures of the plant, are present in the embryo of the seed as primordia and are formed practically simultaneously.

In the embryo of the seed of the sweet clovers the radicle, hypocotyl, cotyledons, plumule, and the crown buds are recognizable. Following the development of the plant from the embryo, the radicle becomes the dominant primary axis of the roots, the hypocotyl enters into the formation of the crown, cotyledons disappear after the seedling stage, the buds in the axils of the cotyledons become the crown buds, and the plumule forms the first-year vegetative shoot.

The seedlings of the sweet clovers are epigeal. The hypocotyl commonly elevates the cotyledons one to two inches above the ground. After a few weeks the roots and hypocotyl begin to contract in length and finally the hypocotyl, crown buds, and the basal portion of the shoot are brought below the surface. This is a notable adaptation, in that the resulting position of the crown buds, usually two to several inches under the ground, is much less hazardous in respect to hard freezing.

In the discussion that follows, the term "root" is used to designate the underground portion of the plant, and thus includes the true root, hypocotyl, and the crown buds.

MATERIALS AND METHODS

This report is limited almost entirely to the biennial white sweet clover, *Melilotus alba* Desr. The data obtained at Ames on *Melilotus officinalis* (L.) Lam. were corroborative of reports from other stations that the growth habits are fundamentally the same as those of *Melilotus alba*. The yellow species is one to two weeks earlier in central Iowa and develops a proportionally heavier root and crown than the white species.

The investigations were conducted at Ames, beginning in 1928 and extending over a period of five years, with an interruption in 1930 due to the excessive drought. All of the soils on which the plants grew were of the drift type and mostly sandy loam. A few plots were on gravelly loam, and a few on alluvial bottom land.

Both the white and yellow species grow wild in abundance in the vicinity of Ames. Areas suitable in size for investigations and bearing pure stands of thrifty biennial sweet clover plants are common along the highways, railroads, and on vacant lots and other lands not under cultivation. On such areas of natural seedings of ordinary wild strains most of the experiments were conducted. In 1929 there was included one cultivated plot on which the plants were grown in rows and kept clean, the object being to minimize competition. The plot was seeded May 20, with Iowa-grown seed. In 1931 the experiments were limited entirely to cultivated plots, two of which were seeded broadcast and the other in rows. These were seeded May 20 and with Iowa-grown seed. In 1932 only natural seedings were used. On plots in central Iowa, where the seeds have wintered as shed from the plants, germination usually occurs the latter part of March or first of April. Plants on the wild plots, therefore, had one month's to six weeks' start of plants on the cultivated plots. The entire range of experiments covered differences in competition, in types of soil, in dates of seeding, and in the climatic conditions of the different years. In size the plots ranged from about 12×36 feet up to $\frac{1}{8}$ of an acre. The wild plots varied considerably, their size depending on how much of the wild stands met the requirements of the experiments. In the study of the effects of the removal of the tops on roots and crowns, plots uniform in stands, size of plants, soil conditions, etc., were required. The mowings on each date covered about one-third of the area of the plots.

In harvesting the samples for weight determinations, an effort was made to include all of the root systems as nearly as could well be done. Excepting in the latter part of the season, the root systems were removed in blocks of soil which, after being pulverized, was shaken from the roots. In the latter part of the season, when the roots of the large plants were generally three to four feet in length, the dirt was removed on one side and loosened on others to a depth of two to three feet, after which the roots were pulled up with relatively little loss. Of course, in all cases much of the finer portions of the roots was lost, and the weights, therefore, only approximate the weights of the entire root systems. These discrepancies, probably in any case not more

than five grams in green weight, are negligible in so far as their consideration would alter the conclusions supported by the weights obtained.

As soon as removed from the soil, the plants were wrapped in canvas to prevent drying and transferred to the laboratory, where on arrival the weighings were made. The tops were first removed and weighed. The roots were washed under the tap, dried with paper towels and by evaporation until free from surface moisture, and then weighed. The defense for this use of the green weights is seen in table 1, which shows that the ratio of tops and roots is practically the same whether green, air-dry, or oven-dry weights are used.

TABLE 1. *Green, air-dry, and oven-dry weights of roots and tops, 100 plants in each determination*

Date of collection	Wt. of roots (grams)			Wt. of tops (grams)			Wt. of roots in percentage of wt. of entire pl.			Wt. of tops in percentage of wt. of entire pl.		
	Green	Air-dry	Oven-dry	Green	Air-dry	Oven-dry	Green	Air-dry	Oven-dry	Green	Air-dry	Oven-dry
Aug. 3	473	138	125	1558	471	442	23	22.6	22	77	77.4	78
Aug. 13	1620	444	399.6	4550	1290	1161	26.3	24.7	24.8	73.7	75.3	75.2
Oct. 13	1780	606	540	1994	724	680	47	45.5	44.2	53	54.5	55.8

Since the consideration of weights was concerned chiefly with ratios, there was not enough difference in favor of the dry weights to warrant the extra outlays in time and labor to obtain them. The three kinds of weights were compared (table 1) in August and also in October, thus both before and well toward the end of the active growth period of the roots. The weights of the roots near the beginning and the end of the active growth period are most involved in the determinations that follow.

A comparison of the data in table 2, where air-dry and oven-dry weights are given in percentages of green weights, shows that the water content of both roots and tops is somewhat less in October, the difference being approximately four and six per cent, respectively. Consequently dry weights would show a greater fall growth than the green weights show. In tables 3 and 4,

TABLE 2. *Air-dry and oven-dry weights in percentages of green weights (based on weights in table 1)*

Date	Air-dry weight in percentages of green weight		Oven-dry weight in percentages of green weight	
	Roots	Tops	Roots	Tops
Aug. 3	29.9	30.2	26.4	28.3
Aug. 13	27.4	28.3	24.6	25.5
Oct. 3	34.0	36.3	30.5	34.0

where weights of roots and tops obtained at intervals through the first season's growth are recorded for comparison, the substitution of dry weights for green weights would show a relatively greater increase in weights in October and November; but inasmuch as weights of roots and tops would be similarly altered, the substitution of dry weights would make no significant changes when the weights of roots and tops are considered as ratios of the weights of the entire plants. By using the percentages in table 2, all the weights in the following tables may be converted into dry weights.

In both 1928 and 1929 use was made of the positive correlation between the weight of the root and the diameter of the basal portion of the stem of the aerial shoot.

The first year the aerial shoot arises from the crown usually as a single stem. The diameter of this stem is directly correlated with size and weight of the root. In October of 1928 three determinations, each based on 50 plants, gave correlation coefficients of $+.98$, $+.91$, $+.88$, respectively, between the weight of the root and the diameter of the stem. In October of 1929 another determination based on 50 plants gave a correlation coefficient of $+.89$. The volumes of roots were at first assumed to be similarly correlated with diameter of the aerial stem, an assumption justified later when ratios of volumes and weights were compared. This close correlation of the weight and volume of roots to the stem diameter was used advantageously in estimating the effects of the removal of the tops on the subsequent root growth. Plants from mowed and unmowed areas were matched as to the size of their aerial stems, and volume or weight of roots was then determined. The differences between the volumes or weights of roots from the mowed and unmowed areas were considered a measure of the effects of the removal of the tops. When determinations based on both volume and weight of 100 pairs of matched plants were compared, the differences between the members of the pairs were slightly greater in most cases when based on weight. The differences, however, did not exceed two per cent and were less than one per cent in most cases. The correlation between volume and weight of roots is, therefore, quite close, and one may be substituted for the other in comparing roots from the differently treated areas.

RELATIVE GROWTH OF ROOTS AND TOPS AT DIFFERENT PERIODS DURING FIRST SEASON

The object of this phase of the work was to disclose the program of growth followed by the roots and the tops through the first season.

The relative growth rates of roots and tops were ascertained from weighings made at intervals through the summer and fall, over a period of three years, 1929, 1931, and 1932. The experiments were planned to include variations that might arise from differences in soil, competition, time of seeding, and seasonal differences of the different years.

In 1929 eight plots, about 12×36 feet, were included. Plots I, II, III,

IV, and V were staked off on some vacant lots on which the stand of white biennial sweet clover was thick and fairly uniform in size. The soil was below the average in fertility, and competition among the plants was strong. Plot VI was on gravelly soil. The plants were also crowded and fairly uniform in size. Plot VII was on fertile alluvial soil and plants were not crowded. Plot VIII was a cultivated plot on which the plants were in rows and given ample space for good growth. This plot was seeded May 20. The natural seedings in 1929 germinated the latter part of March and forepart of April. The plants on the cultivated plot, therefore, were six weeks or more later than the natural seedings in getting started. At each sampling 100 plants were taken as dug. Although most of the 100 plants ranged pretty closely around a certain size, various sizes down to very small plants were present and were included in the determinations. The data obtained from these plots are given in table 3.

A comparison of the weights obtained at different dates shows the following: (1) that the plants are 80 per cent or more tops during the early part of the season but more than 50 per cent root at the end of the season; (2) that both tops and roots gain in weight up to the latter part of August or early in September with the greater increase in weight in case of the roots; (3) that during September and the remainder of the growing season the tops generally make very little growth, while the roots have a marked increase in growth, especially during September; (4) that differences in time of seeding, in competition of plants, types of soil, and vigor of growth, so far as they pertain to these experiments, did not alter the general characteristics of the season's growth.

The differences in the growth rates of tops and roots at different periods in the season are graphically shown in figure 1. These curves show a marked increase in weight of roots during September on all the plots but in weight of tops only a little increase and on some plots a reduction in weight. On plot VIII, where the plants were late in getting started but favored in opportunities for good growth, the tops continued to increase in weight during September. This fact may be due partly to the late start in growth on this plot, necessitating the prolongation of the growth period in the fall to compensate for time lost in the spring; and it may be a matter chiefly of extraordinary vigor, an explanation supported by a similar prolongation of growth of tops on plot VII. However, despite the prolongation in growth of tops on plots VII and VIII, the pronounced increase in root growth occurred in September, thus conforming to what occurred on other plots. On plots I, IV, and V, the increase in rate of growth of roots was maintained through October, whereas on the other plots there was a marked drop in root growth after September. The prolongation of growth of roots in the fall is not uncommon in plants that have grown slowly during the previous part of the season.

In 1931 three plots were planted on May 20 to biennial white sweet clover. Two of the plots were planted in rows and plants cultivated. The third plot

TABLE 3. *Determinations in 1929 of the relative growth rates of roots and tops based on green weights of 100 plants taken as dug. Weights in grams.*

Date of determinations	Plot	Weight of entire plants	Weight of tops	Weight of roots	Weight of tops in percentage of total wt.	Weight of roots in percentage of total wt.	Average weight of tops per plant	Average weight of roots per plant
July 2 and July 3	I	305	260	45	85	15	2.6	.45
	II	264	210	54	80	20	2.1	.54
	III	660	575	85	87	13	5.75	.85
	IV	640	515	125	80	20	5.15	1.25
	V	575	455	120	80	20	4.55	1.20
	VI	610	510	100	84	16	5.1	1.00
	VIII	605	515	90	85	15	5.15	.90
	Average		434	88	83	17	4.34	.88
Sept. 1-4	I	1561	1195	380	76	24	11.95	3.80
	II	1883	1425	460	75	25	14.25	4.60
	III	780	603	175	77	23	6.03	1.75
	IV	1150	750	400	65	35	7.50	4.00
	V	800	467	333	59	41	4.67	3.33
	VI	1000	656	254	75	25	6.56	2.54
	VII	2155	1355	800	63	37	13.55	8.00
	VIII	4690	3300	1390	70	30	33.00	13.90
	Average		1220	524	77	33	12.20	5.24
Oct. 2-6	I	2066	1227	828	60	40	12.27	8.28
	II	2733	1183	1350	50	50	11.83	13.50
	III	989	492	497	50	50	4.92	4.97
	IV	933	470	463	50	50	4.70	4.63
	V	1181	516	665	44	56	5.16	6.65
	VI	1692	694	908	41	59	6.94	9.08
	VII	3100	1700	1400	55	45	17.00	14.00
	VIII	8000	3556	4444	44	56	35.56	44.44
	Average		1232	1331	49	51	12.32	13.31
Nov. 4-8	I	1782	542	1240	30	70	5.42	12.40
	II	1492	542	950	36	64	5.42	9.50
	III	722	230	492	32	68	2.30	4.92
	IV	1227	409	818	33	67	4.09	8.18
	V	1350	483	967	30	70	4.83	9.67
	VI	1483	580	900	39	61	5.80	9.00
	VII	2450	1025	1425	42	58	10.25	14.25
	VIII	5020	1670	3350	33	67	16.70	33.50
	Average		584	1255	44	66	6.85	12.55

was broadcast and the stand obtained was thick. All the plots were on fertile sandy loam and the growth was above the average. The character of the growth was practically the same on the three plots. The data for 1931, in table 4, pertain to one of the plots seeded in rows but are typical for the other plots. In procuring the samples for the determinations in 1931 and 1932, the plants were taken as dug without regard to a specified number.

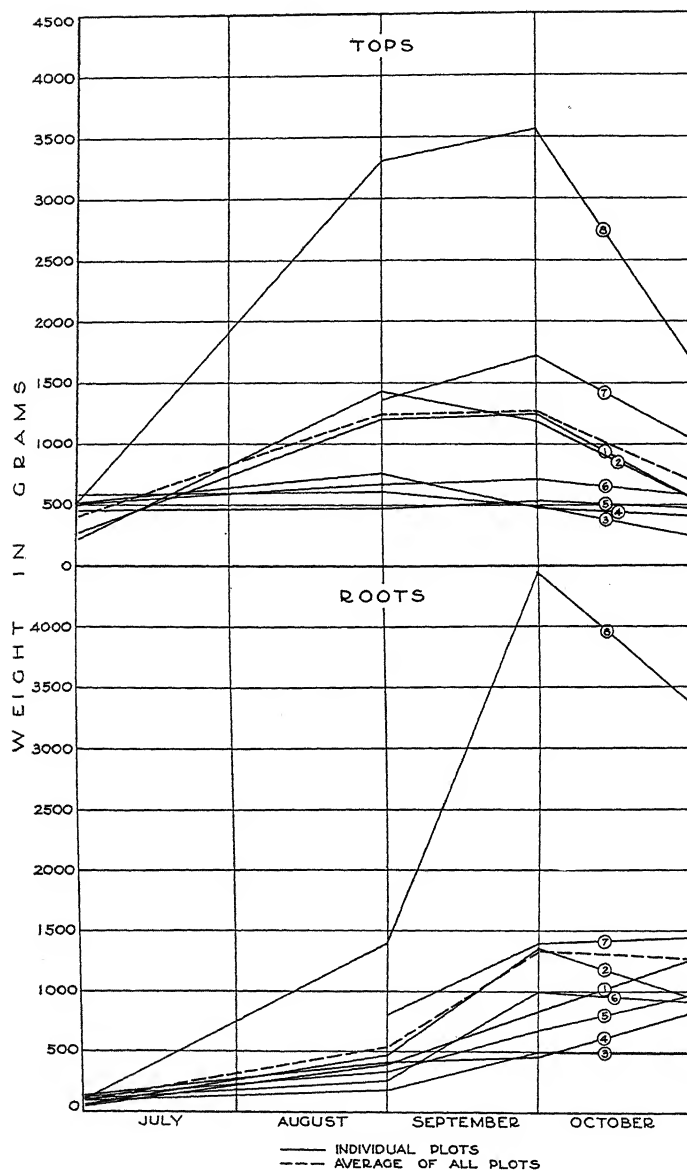


Fig. 1. Relative growth of roots and tops on eight different plots in 1929, as shown by actual weights of roots and tops determined at intervals through the first season.

In 1932 the investigations were limited to one plot of about $\frac{1}{8}$ of an acre of natural seeding. The soil was sandy loam and average in fertility. The plants were vigorous, partly because of the favorable rainfall of the season. The plants were crowded but noticeably uniform in stand and size. The data pertaining to this plot are included in table 4.

TABLE 4. *Relative growth of roots and tops of biennial white sweet clover during the first growing season*

Date of determinations	Number of plants	Total weight of plants (grams)	Weight of tops (grams)	Weight of roots (grams)	Weight of tops in percentage of total weight of plants	Weight of roots in percentage of total weight of plants	Average weight of tops per plant (grams)	Average weight of roots per plant (grams)
1931								
June 4	169	255.5	231	24.5	90.5	9.5	1.4	.15
July 3	93	650	532	118	81.4	18.6	5.7	1.3
Aug. 4	187	2350	1920	430	80.2	19.8	10.3	2.2
Sept. 3	132	1570	1122	448	72	28	8.5	3.3
Oct. 3	111	3097	1488	1609	47	53	13.97	14.5
Nov. 6	174	4163	1793	2388	43.3	56.7	10.3	13.6
Nov. 8	60	1110	190	990	19	81	3.6	15.3
1932								
June 25	140	734	674	64	91.8	8.2	4.8	1.16
Aug. 9	165	2251	1739	512	77.2	22.8	10.5	3.1
Aug. 28	84	1624	1161	461	71.5	28.5	13.8	5.5
Oct. 3	172	3410	1501	1908	44	56	8.7	11.3
Oct. 20	90	1455	750	705	51	49	8.3	7.7
Nov. 1	100	4028	1820	2180	45.1	54.9	18.20	21.80

The data for 1929, 1931, and 1932 are remarkably uniform in the characteristics of growth shown. The particular features characteristic of the growth habits of biennial white sweet clover in 1929, as pointed out in the discussion of table 3 and in figure 1, are repeated in 1931 and 1932. The growth in each of the years is characterized by the following: a continuous increase in weight of both tops and roots up to the forepart of September, with a greater acceleration of growth in the case of the roots; a decided drop in the growth of tops during September and the remainder of season; a pronounced increase in the growth rate of roots during September; and, excepting in 1931, a marked reduction in growth of roots after September. These features are more vividly portrayed in figures 2 and 3. In figure 3, where the increase in the weight of the tops and roots is reckoned in percentages of the weights of the entire plants, the curves are very similar. The exceptional growth of tops and more especially that of the roots shown during October of 1932 could have been due partly to the favorable growth conditions of the fall, but most of it was probably due to poor sampling. The discrepancy between weights obtained on October 20 and November 1 and the fact that the ratio of the weights of the tops and roots conforms to that of other years support such an explanation.

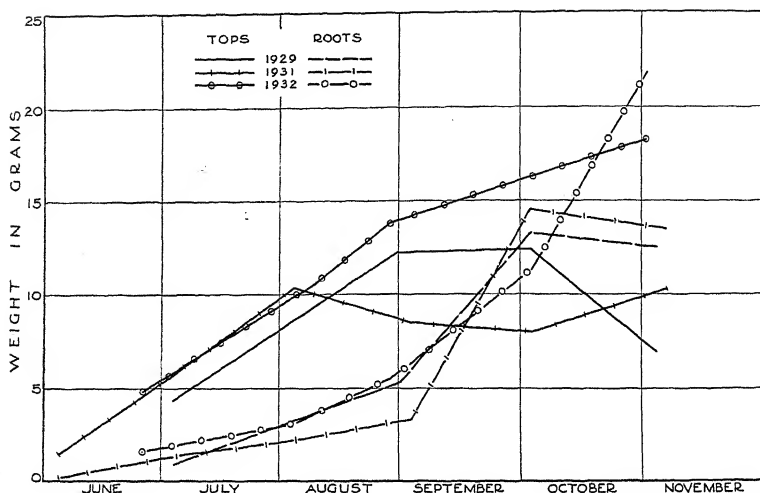


Fig. 2. Relative growth of roots and tops during the three years 1929, 1931, and 1932 as shown by the actual weights of roots and tops at different intervals.

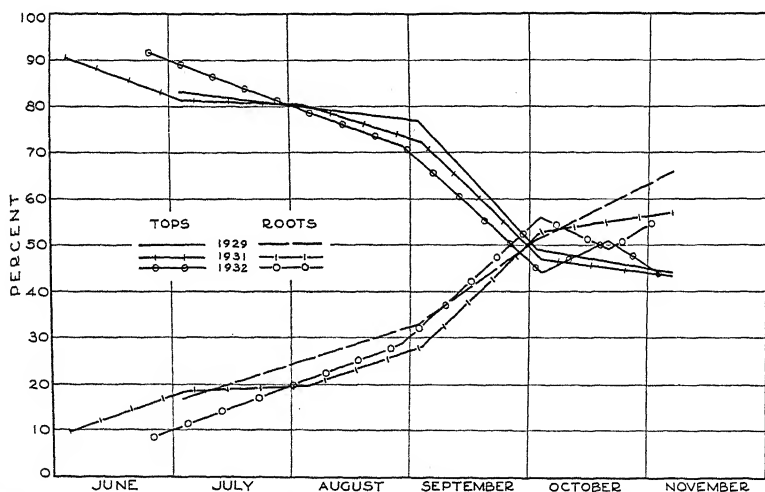


Fig. 3. Relative growth of roots and tops as shown by considering the weights of roots and tops as percentages of the weights of the entire plants.

THE EFFECT OF THE REMOVAL OF THE TOPS ON THE SUBSEQUENT GROWTH OF THE ROOTS

Both general observations and experiments in growing the biennial sweet clovers have shown that mowing near the beginning of the special growth period of the roots is apt to have serious effects on the subsequent growth of the roots. As shown by the data on the relative growth of roots and tops, the period of rapid root growth begins in the locality of Ames near the first of

September. In accordance with this information, the tops were removed August 28 in 1928 and early in September in 1929, 1931, and 1932. The tops were removed with a scythe or sickle and cut low enough, usually within three or four inches of the surface, to include all or nearly all of the leaves. In the same years, excepting in 1928, the tops were removed from another area of the plots in early October, the object being to ascertain also the effects of mowing after the roots had passed the special growth period. In 1928 the work was limited to one plot of about one-eighth of an acre of natural seeding, selected for its uniformity in the stand and in the size of plants. In 1928 the effect of mowing on the subsequent growth of roots was estimated by comparing the volumes of roots of plants from the mowed and unmowed areas. The selection of plants for the comparison was based on the diameter of the stem just above the crown. Plants from one area (either the mowed or unmowed) were matched with plants approximately equal in diameter of stem from the other area. The volumes of the roots of the pairs of matched plants were estimated from their displacement of water in a graduate. To show the nature of the results, the determinations for 25 pairs of matched plants are given in table 5.

TABLE 5. *Effect of mowing in 1928 upon subsequent growth of roots as shown by a comparison of the volumes of roots of mowed plants with volumes of roots of plants not mowed*

Number of the determination	Not mowed	Mowed Aug. 28	Volume of roots of mowed plants in percentage of volume of roots of plants not mowed
1	12	5	42
2	16.25	4.75	29
3	18	5	28
4	16	5.25	33
5	6	4	66
6	16	13	80
7	16	7	44
8	8	9.5	119
9	12	2	17
10	5.5	4	70
11	18	19	106
12	7	2	29
13	5	3	60
14	5.75	3	52
15	19	6	32
16	20	17.5	87.5
17	21	7	33
18	4.5	4	90
19	7.5	4	53
20	5	2	40
21	4	3.5	87.5
22	15	13	86
23	14.5	5	31
24	17	8	47
25	10	6	60
Average			56

The average volume of the roots of 200 plants from the area mowed August 28 was 51 per cent of the average volume of the roots of corresponding plants from unmowed area. Although there is a wide range in the variations in table 5, a decided effect of mowing is shown.

TABLE 6. *Comparative weights of roots of plants with stems approximately equal in size from 1929 plots (each estimate based on 100 matched pairs)*

Plot	Weight not mowed	Mowed October 4		Mowed September 4	
		Weight (grams)	Wt. in percentage of wt. of not mowed	Weight (grams)	Wt. in percentage of wt. of not mowed
I	933			470	50
II	1050			406	40
III	586	643	109	286	49
IV	961			700	72
VI	1521	1420	93		
	Average		101		52

In 1929, as shown in table 6, weight was substituted for volume as a basis of comparison, and on two plots the effect of mowing in early October was included. The data in table 6 show that the average growth of roots on areas mowed September 4 was 52 per cent of that on the unmowed areas, whereas

TABLE 7. *Effect of mowing as shown by a comparison of the weights of the roots of 100 plants taken as dug from mowed and unmowed areas of plots with stand and size of plants approximately uniform at time of mowing*

Plot	Wt. of roots of unmowed plants (grams)	Mowed September 1-4		Mowed October 1-4	
		Wt. of roots (grams)	Wt. of roots in percent- age of wt. of roots of unmowed plants	Wt. of roots (grams)	Wt. of roots in percent- age of wt. of roots of unmowed plants
<i>1929 plots. Determinations, Dec. 10-12, 1929</i>					
I	880	552	62.7	1000	113
II	889	492	55.4	820	92
III	330	152	40.6 —	340	103
VI	1300	625	48	1400	107
Average	850	455	53.5	890	104.7
<i>1931 plots. Determinations in April, 1932</i>					
I	8200	3600	44	7800	95
II	1610	1090	68		
III	3620	2600	72	3600	99
Average	4476	2430	53	5700	97
<i>1932 plots. Determinations, Nov. 5, 1932</i>					
I	523	275	52.8	468	89

the average growth of roots on areas mowed October 4 was practically the same as that on the unmowed areas. Evidently the roots on the areas mowed October 4 had practically completed their growth during September and consequently were not much disturbed by the removal of the tops.

A second set of determinations, based on the comparative weights of the roots of 100 plants taken as dug from each of the areas, was made on four of the 1929 plots. This method assumes that the plants were uniform in size on the different areas at the date of the first mowing. The results obtained (table 7) confirm those in table 6 and therefore add confidence in the results based on correlation of volume or weight of roots with diameter of stem. The determinations in 1931 and 1932, also included in table 7, are closely corroborative of the results obtained in the previous years.

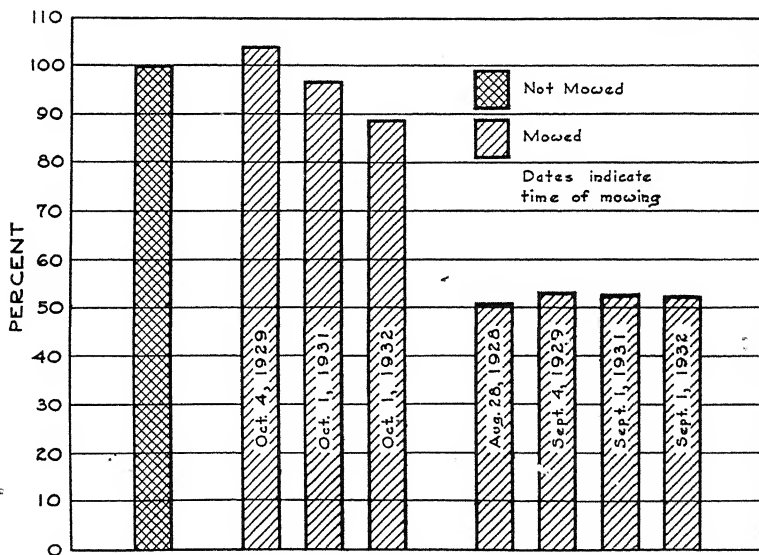


Fig. 4. Effect of the removal of the tops on subsequent growth of the roots as shown by the comparative weights of roots on mowed and unmowed areas, the weight on the unmowed areas being considered 100 per cent.

The results obtained on the effects of mowing on the subsequent growth of roots are summarized in figure 4, where growth on mowed areas is given in percentages of the growth on unmowed areas, the growth on unmowed areas being considered 100 per cent. According to this figure, the growth of roots is very much hindered by the removal of the tops at the beginning of the special growth period of the roots, the roots being able to attain only about 50 per cent of the weight of the roots of unmowed plants, whereas in the case of mowing early in October, which is near the end of the special growth period, there is practically no interference with growth.

THE EFFECT OF THE REMOVAL OF TOPS ON THE WINTER
RESISTANCE OF ROOTS

The mowed and unmowed areas on some of the plots were further investigated as to the effect of the removal of the tops on the ability of the roots to endure low temperatures. Naturally so much disturbance in root growth as follows when the tops are removed near the first of September can be expected to have considerable influence on the winter-resistance of the roots.

The effect of the removal of tops on the winter-resistance was estimated by a comparison of the mortalities on the mowed and unmowed areas. On the 1928 plot estimates were made April 12 and June 8, 1929. On April 12, the determinations were based on counts of both dead and live plants on four equal quadrats on each of the areas. The results, recorded in table 8, show

TABLE 8. *Effect of mowing on winter-resistance as shown by the relative mortalities on the differently treated areas*

Unmowed area			Area mowed August 28		
No. of quadrats	No. of plants	Percentage of dead plants	No. of quadrats	No. of plants	Percentage of dead plants
1	150	3.3	1	183	56
2	80	0	2	70	59
3	104	0	3	76	53
4	128	3	4	138	60
Average		1.5	57.5		

that mowing August 28 on the 1928 plot was responsible for more than 50 per cent mortality up to April 12. Many more of the plants succumbed later as shown on June 8, 1929, when counts were made of plants still alive on 10 equal quadrats distributed over each of the areas (table 9). The plants

TABLE 9. *Effect of mowing as shown by a comparison of the number of plants alive on the differently treated areas on June 8, 1929*

Plot not mowed		Plot mowed August 28, 1929		
No. of quadrats	No. of plants	No. of quadrats	No. of plants	Plants on mowed plot in percentage of those on plot not mowed
1	61	1	22	
2	68	2	3	
3	80	3	10	
4	38	4	22	
5	56	5	2	
6	58	6	12	
7	65	7	8	
8	82	9	13	
10	48	10	0	
Average	63		10	16

still alive on mowed areas were only 16 per cent of those on the unmowed areas and were, on the average, much smaller and lacking in vigor. The mowing on August 28 resulted in practically clearing that portion of the plot of sweet clover, whereas the unmowed portion produced a good crop of vigorous plants the second season. The effects were probably extreme on the 1928 plot, as the winter of 1928-29 was rather severe due to the absence of snow during some periods of hard freezing.

In 1929 and 1931 the study was extended to include the effects of mowing in October. In 1929 four plots were involved, one of which (plot VI) was located in the depression of an abandoned gravel pit where there was protection from winds, whereas the other three were located on open prairie. The determinations on the 1929 plots were made between December 10 and 20 after there had been some hard freezing. Each area involved in the comparative study was represented by 100 plants taken without selection and transferred to the greenhouse, where they were provided with favorable conditions for growth. Plants injured so severely as barely to survive were considered dead. The results are recorded in table 10. The table shows a range from

TABLE 10. *The effects of mowing on the winter-resistance of roots on plots of 1929 as shown by determinations in December*

Plot	Area not mowed		Area mowed Sept. 4		Area mowed Oct. 4	
	Live	Dead	Live	Dead	Live	Dead
I	94	6	54	46	93	7
II	97	3	60	40	98	2
III	96	4	40	60	95	5
VI	98	2	86	14	96	4
Average	96	4	60	40	95.5	4.5

14 to 60 per cent mortality on areas mowed September 4. The lowest mortality was on the plot in the gravel pit. The mortality on the unmowed areas ranged from 2 to 6 per cent. The mortality on the areas mowed October 4 ranged from 4 to 7 per cent, with the average nearly the same as that of the unmowed areas. Due to unavoidable circumstances, determinations later in the winter were not made on 1929 plots, but as in case of the 1928 plot, it is quite probable that later determinations would have shown a more pronounced effect of the mowing on September 4. The feature of special interest is that mowing on September 4 very much lowered the winter-resistance of the roots, whereas mowing on October 4 had practically no influence on the winter-resistance of the roots.

On the plots of 1931 the determinations were made in the spring of 1932 just after the second season's growth had begun and were based on percentage of dead plants estimated from counts on equal quadrats (table 11). As the table shows, there was not much winter-killing on either the mowed or unmowed areas on the plots of 1931. The winter of 1931-1932 was quite mild.

TABLE 11. *Effect of mowing on winter-resistance as shown on 1932 plots*

Plot	Percentage of dead plants		
	Unmowed area	Mowed September 1	Mowed October 1
1	4	15	8
2	8	20	10
3	6	18	5
Average	6	17	7

Some freezing in the latter part of winter caused considerable heaving which, in most cases, the plants were able to survive. The effects of mowing on winter-resistance are summarized in figure 5.

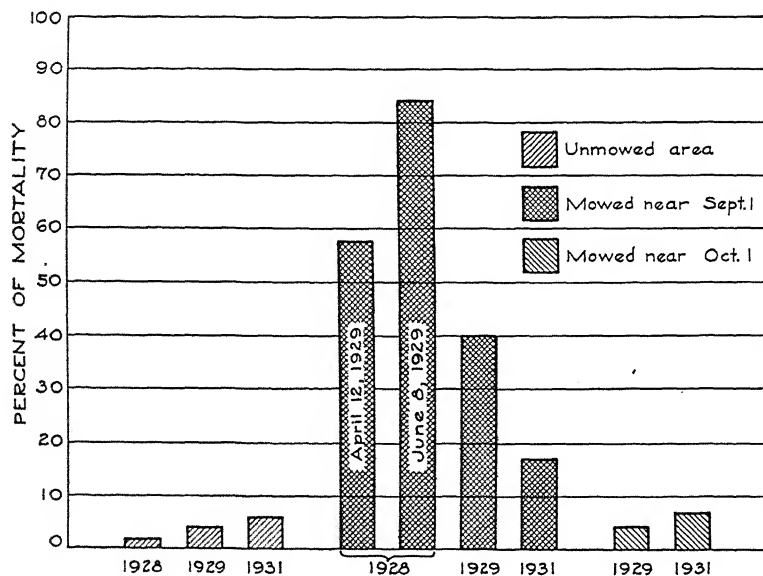


Fig. 5. Effects of mowing near September 1 and October 1 on the winter-resistance of roots, as shown by comparing the mortality on the differently treated areas.

Mowing at the beginning of the special growth period of the roots not only prevented the normal increase in size but interfered with the normal storage in the roots and stimulated in a large percentage of the plants the growth of the crown buds. Starch tests showed very little or no starch in roots of plants mowed at the beginning of the period of the special growth of the roots, whereas roots from unmowed areas were heavily loaded with starch. The absence of starch can be attributed partly to the depletion through the extra growth of crown buds but chiefly to the absence of the tops, which apparently devote their energies especially to the growth and storage in the roots during the special period of root growth.

SUMMARY

The investigations, covering four years, set out to ascertain (1) the natural features that characterize the first-year growth of the biennial sweet clovers growing under central Iowa conditions and (2) the relation of the first-year growth features to the effects that follow the removal of the tops at certain stages in the first season's growth. The data recorded pertain to the white species, *Melilotus alba* Desr., but with slight modifications they are also applicable to the yellow species, *Melilotus officinalis* (L.) Lam.

The material included both wild and cultivated plots, so selected and managed as to afford differences in soils, thickness in stand of plants, and in dates of seeding.

The first season's growth was characterized by certain inherent features that persisted regardless of the differences in time of seeding, types of soil, amount of competition, and seasonal differences. The characteristic features were: During the first two or three months of the first season's growth the emphasis was on the growth of the tops, the weight of which attained to 90 per cent or more of the weight of the entire plant; during the remainder of the season the emphasis was on root growth, which was characterized by a gradual increase in rate up to about September 1 and then by a marked increase in rate that continued through September and sometimes into early October before it subsided; about the time the period of pronounced root growth began, top growth very much slowed down or ceased entirely; the roots during their special growth period more than doubled their weight, increasing from 25 to more than 50 per cent of the weight of the entire plant.

The removal of the tops at dates just previous to or at the beginning of the special growth period of roots and crowns (the latter part of August or early in September) inhibited subsequent growth and was followed by low winter-resistance and also by poor growth the second season of plants that survived the winter.

On the other hand, the removal of the tops after the special growth period was over—any time after September—had little or no detrimental effect.

The growth of roots and crowns during their special growth period was accompanied by a noticeable enlargement of the crown buds, as was seen by comparing crowns at different times in the growth period.

These disclosures concerning the growth habits of the biennial sweet clovers at the Iowa Station agree with those reported by Willard of the Ohio Stations. Willard also shows that the biennial sweet clovers followed the same program of growth at the Ohio Stations when subjected to the disturbances attendant on a nurse crop. The effect of the nurse crop was mainly a retardation of about a month in the occurrence of the growth stages.

Since cultural practices hinge so much on the special growth period of roots and crowns, the date of the occurrence of this event is a matter of considerable concern in every locality where the biennial sweet clovers have a place among the cultivated crops.

At present the determination of the date of the special growth period of roots and crowns is to a large extent a local problem. The data available are not adequate as a basis upon which estimates or predictions can be made concerning the extent of the area over which the observations of any particular station can be applied.

Considerable variation in the date of the special growth period in connection with differences in latitude are to be expected, for there is evidence that changes in light and temperature relations have much to do with the shifts in growth the plants make as the end of the season approaches.

Willard shows that at Columbus, Ohio, about 2 degrees south of the Iowa Station, the special growth period occurred during September when no nurse crop was used, thus corresponding in date with the special growth period at the Iowa Station. Furthermore, data taken in 1929 at the Kentucky Station at Lexington, about three degrees south of the Iowa Station, show that the special growth period of roots and crowns occurred there mainly during September, thus coinciding in time of occurrence with the special growth period at Columbus, Ohio, and at the Iowa Station. These three stations constitute the points of a triangle with a base of more than ten degrees of longitude and an altitude of about three degrees of latitude. This triangle comprises a large part of the sweet clover area. Not all reports, however, show such close agreement. For instance, in connection with the reports from the Ohio stations it is shown that on two plots on the Clermont County Farm (about 100 miles south of Columbus) the special growth period was about a month later than at Columbus. This discrepancy seems too large to be attributed entirely to the difference in latitude between the two stations.

The crown buds are fairly reliable indicators of the progress of the special growth period of the roots, a fact pretty generally known by growers of sweet clover. At the beginning of the special growth period of the roots, the crown buds are quite inconspicuous, since during the previous part of the growing season they are almost dormant. Along with the acceleration in the growth of the roots there occurs an acceleration in growth of crown buds which consequently soon become noticeably enlarged. A familiarity with the changes in the crown buds during the different stages in the plant's growth makes it possible to recognize and follow the progress of the special growth period of the roots and crowns.

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DENDROGRAPH STUDIES ON *ACHRAS ZAPOTA* IN RELATION TO THE OPTIMUM CONDITIONS FOR TAPPING

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It is the common practice of chicleros in the chicle forests of Southern Mexico and Central America to bleed *Achras zapota* in the early morning hours before sunrise or as shortly thereafter as possible. They have discovered from long practice that a greater yield is to be secured in the morning when relative humidity, temperature, and rate of transpiration are most favorable for the flow of latex. It is quite obvious, however, in such tropical regions that immediately after sunrise, with an increase in temperature, wind velocity, rate of transpiration, and consequent loss in relative humidity and tree turgidity, there is a marked tendency for the flow to lessen and the thick latex to coagulate in the cuts, and thus prevent the maximum yield that might be available under more constant ideal tapping conditions. This fact led us during the 1931-32 chicle season to consider for experimental as well as commercial purposes the possibilities of tapping at some other time of the day when external environmental factors are less variable.

In attempting to determine the most favorable time for tapping, it soon became apparent that one of two relatively simple methods of experimentation might be employed: extensive bleeding experiments at regular time intervals of the day; or determination of the optimum weather conditions for bleeding and their effect on the sapodilla tree by delicate recording instruments and correlating these effects with yield. In view of the fact that the results from the first method would be difficult to interpret in relation to the constantly changing weather conditions, and also because it involves a tremendous outlay of experimental trees, time, labor, and money, the latter method was resorted to—particularly since it has been repeatedly demonstrated by MacDougal (1921, 1924, 1925, 1930), Korstian (1921), Pearson (1924), Lodewick (1925), Haasis (1932), and others that trees ordinarily undergo more or less rhythmic diurnal expansion and contraction in diameter, which appear to be due primarily to turgidity changes in relation to the prevailing rainfall, relative humidity, sunshine, temperature, and other weather conditions. For these reasons and on the basis of tapping data from previous years it was assumed that such changes in the trunk are correlated with and in a measure determine the readiness with which the latex flows from the

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severed laticiferous vessels. Self-recording wind and rain gauges, barometers, thermographs, and hygrographs record the changes in weather conditions, while dendrographs measure fairly accurately the effect of these changes on the tree trunk. No instrument is available, however, for recording the rate and amount of transpiration of the crown of large trees, which are undoubtedly most significant factors in relation to tapping and dendrography.

These dendrograph studies were made at Tower Hill in northern British Honduras during 1931 and 1932. The Tower Hill Estate embraces slightly more than 11,000 acres in the Orange Walk District and lies approximately 25 miles in a straight line inland from the Caribbean Sea. Part of the estate is traversed by a large savannah which connects directly with the sea, while other portions are as much as 20 feet above sea level. The area bordering the savannah is covered by a fairly high and dense jungle, but the remaining parts comprise what is commonly known as "low bush" interspersed with acache. Swamps, savannahs, and "pans" are quite numerous throughout and become filled with water during the rainy season. The estate is bounded on the northwest by Honey Camp Lagoon, which is approximately $1\frac{1}{2}$ miles long and $\frac{1}{2}$ mile wide, and varies from 5 to 30 feet in depth. The eastern part of Tower Hill consists of a fairly open pine ridge or forest, which varies from 4 to 10 miles in depth.

The soil in this region is characteristic of that of northern British Honduras in general. The surface soil varies only from a few inches to several feet in depth and lies on a hard marl and limestone pan. As will be noted below in the description of the individual trees, this limestone may extend to the surface in certain localities, while in the cohune ridges a rich humus may be present to a depth of from 3 to 5 feet. As a result of this characteristic soil formation, very few of the various tree species have a definite tap root which penetrates the limestone pan. Instead they send out lateral roots which extend for great distances immediately below the surface soil.

As to climatic conditions, there are two well-marked seasons in British Honduras. The rainy season usually begins during the latter part of May and early June and continues with some interruptions until January. During this season the wind is prevailing from the south and southeast, except for November and December. These months are quite cool and fairly dry, with the wind in the north and northwest, and as a result there is usually a short fairly dry period in the so-called rainy season. The dry season generally begins in January and extends to the latter part of May and early June. Little or no rain falls during this season, and most of the swamps, pans, savannahs, and small streams dry up, while the soil becomes very parched and cracked. This is usually the season of highest daily temperature, with the wind prevailing in the south and east.

In these studies dendrographs and weather-recording instruments were set up in various parts of Tower Hill Estate. In view of the heavy rainfall and wind during the wet season it became necessary to build small palm huts

around the experimental trees and above the instruments, as is shown in the accompanying photographs below of the individual trees. These huts not only prevented excessive wetting of the graphs but also served to reduce to a minimum the possibilities of expansion and contraction of the instrument metals from exposure to tropical sun, wind, and rain. Sapodilla trees in various parts of the jungle were selected for experimental purposes. As will become more apparent in dealing with individual cases, some trees were out in the open at the edge of a large lagoon and thus completely exposed to wind, sun, and rain, while others were deeply hidden in the jungle. In this manner it was possible to secure data from trees under a wide range of environmental conditions.

OBSERVATIONS AND RESULTS

Tree A

This tree stands in compartment 5, block III, along the fire line separating the open pine ridge from the high bush on the west. It is thus fairly well exposed on the northeast and east to the sun and wind. The sun strikes the bole in the early morning, and the crown is never overshadowed by other

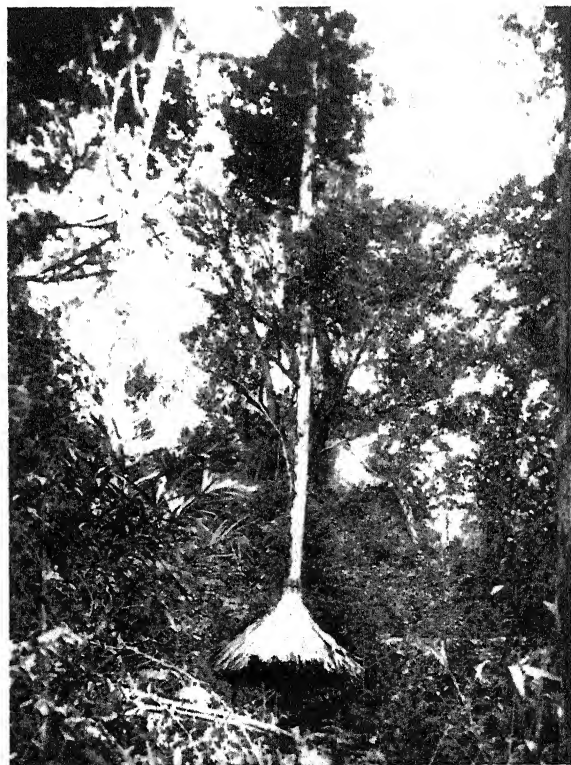


Fig. 1. The relation of tree A to the surrounding trees on the west.

trees until the sun begins to sink in the late afternoon. Its position relative to surrounding trees and undergrowth is well shown in figure 1. The tree itself is fairly small with a shoulder-high girth of less than 3 feet, approximately 50 feet high, and has a straight, smooth bole and a good crown. Its yearly increase in girth has been measured since 1924, and an accurate account of its growth rate over this period is known. Because of this fact tree A has been used for our permanent dendrograph set-up which has now been running continuously for more than a year. The soil in this location is a sandy loam to a depth of approximately 2 feet, and beneath lies a deep compact stratum of marl or white limestone.

In figure 2 is shown the dendrograph record of this tree's daily expansion

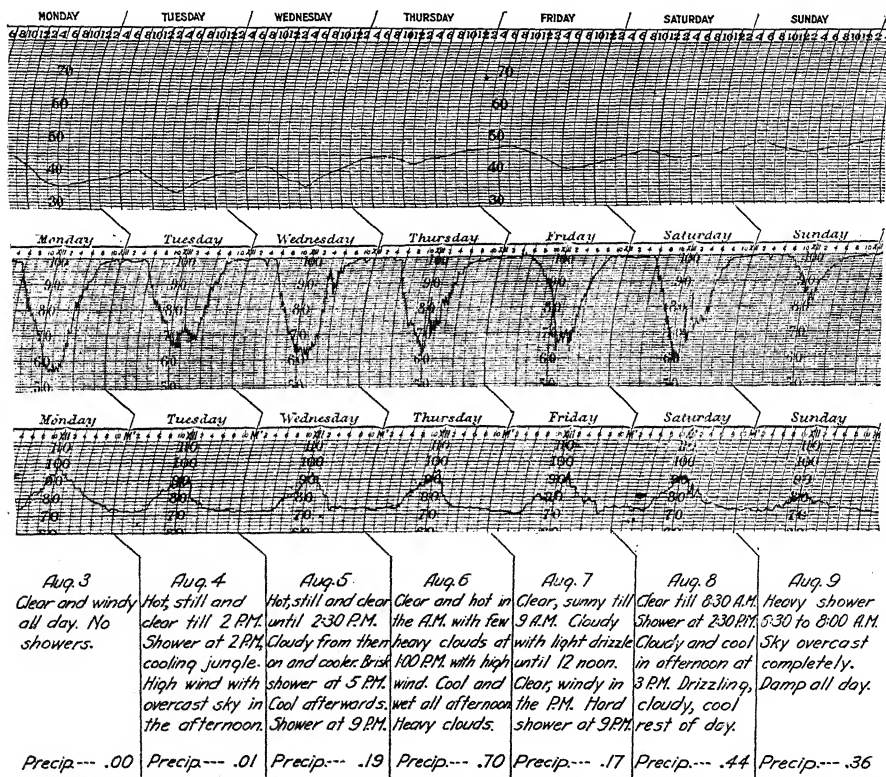


Fig. 2. The weather notes and dendro-, hygro-, and thermograph records of tree A for week ending August 9, 1931.

and contraction for the week ending August 9, 1931. The curve is rhythmic to a marked degree, showing increasing expansion of the trunk during the night and contraction during the day. This curve also shows that the trunk usually reached its maximum diameter between 6:00 and 7:00 a.m. and contracted to a minimum at approximately 5:00 p.m. Such regular rhythmic

changes, however, obtain only under fairly constant weather conditions. A heavy rain, increased humidity, marked changes in temperature, and wind velocity may alter the rhythm considerably, particularly during the day. With the view of presenting more objectively this correlation with weather conditions, in this same figure are shown the temperature and relative humidity records as well as notes on the type of weather for each day. A careful study and comparison of these records and notes show a marked correlation between trunk diameter and weather conditions. On Thursday, August 6, for instance, a hard shower fell at 1:00 p.m. and was followed by a wet and cool afternoon with a heavily overcast sky. The thermo- and hygrograph records show a precipitous decrease in temperature and increase in relative humidity. Accompanying this change in weather, the tree trunk began to expand at approximately 2:00 p.m., and the dendrograph curve thus shows a marked rise. Somewhat similar weather conditions were prevalent on Saturday, August 8, with the result that the dendrograph curve is somewhat higher but less precipitous for that day. On Sunday heavy showers fell early in the morning, and the sky was completely overcast during the day, with a corresponding high mean relative humidity and low temperature. The effect on the tree is strikingly reflected in the dendrograph record of that day. The curve is higher, more even, reaches a maximum at about 8:00 a.m., and does not begin to contract until the late forenoon.

The effect of rainfall, high humidity, and low temperature here shown by the sapodilla tree in British Honduras is strikingly similar to that recorded by MacDougal (1921), Haasis (1932), and others on trees in the temperate zone. MacDougal (1921) found that irrigation of a live oak during a dry period produced a marked increase in diameter of the stem within two hours after the addition of water to the soil. A similar effect is shown on *Achras zapota* by the 7-inch shower at 1:00 p.m., August 6. The dendrograph curve shows almost immediate cessation of shrinkage and a decided increase within an hour.

Tree 2

This tree stands isolated in the camp clearing on the edge of Honey Camp Lagoon and is thus openly exposed on all sides to changes in weather conditions. During the day the wind usually sweeps across the lagoon and strikes the tree with full force, and it is completely exposed to the sun from 7:00 a.m. to 5:00 p.m. The tree stands on a small bank about 12 feet high and is thus some distance from the actual water edge. In relation to its environment it is thus at the extreme range of exposure of the sapodilla trees studied. The surface soil is not much over a foot deep, and the sub-soil consists of a mixture of marl and rock.

In figure 3 are shown the dendro-, thermo-, and hygrograph records of this tree for the week ending September 13, together with weather notes and rainfall. The straight line above the dendrograph curve represents a check

on whether or not the drum carrying the graph was level. It is quite obvious that if the drum were slightly inclined from the horizontal plane the curve would show a steady increase or decrease according to the angle made with the recording pen. For this reason, the pen was raised and the drum twirled around to check the plane of the drum at the beginning of each week's records. A straight line thus indicates an exact horizontal position of the drum. The results here shown are the most striking of any yet obtained and

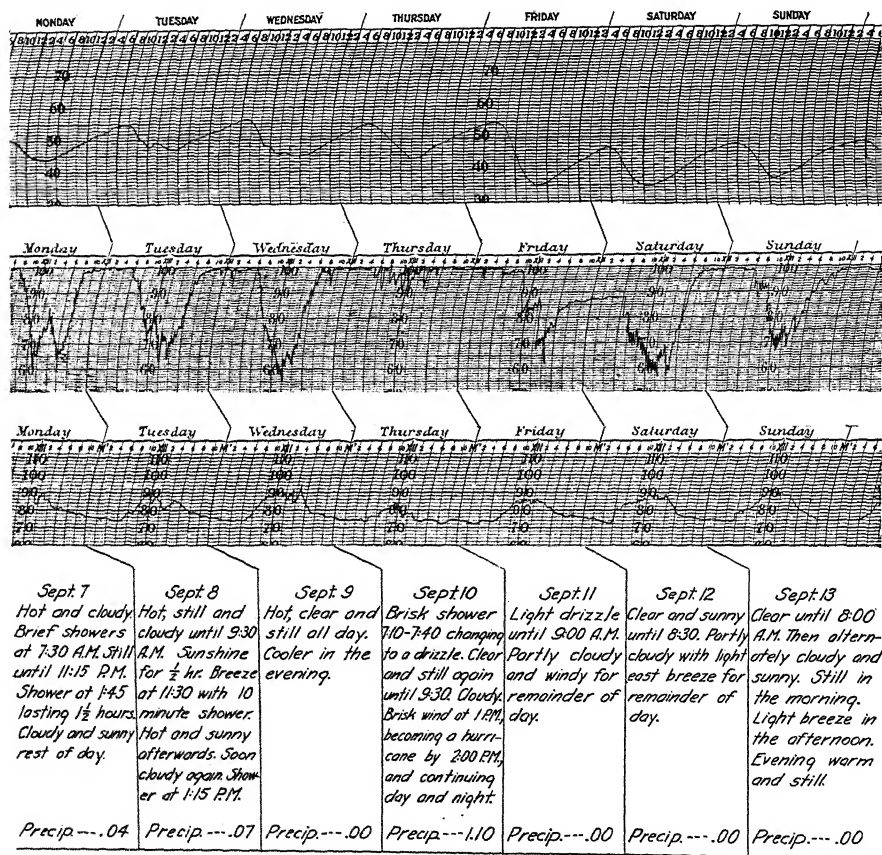


Fig. 3. The weather notes and dendro-, hygro-, and thermograph records of tree 2 for week ending September 13, 1931.

indicate clearly the effect of complete exposure to varying weather conditions on the expansion and contraction of the trunk. The dendrograph curve has an unusually wide daily range of variation, a minimum of 9 mm. on Tuesday and a maximum of 19 mm. on Friday. It parallels to a remarkable degree the relative humidity curve. Two days of this week are particularly significant as far as weather is concerned. On Tuesday brisk showers fell at 11:00

a.m. and 1:15 p.m., cooling the temperature, raising relative humidity, and thus cutting down transpiration. The effect is readily perceptible within a short time thereafter in the dendrograph curve. On Thursday, September 10, occurred the well-known Belize hurricane, which was extremely destructive of human lives and property. Its effect was also strongly felt at Tower Hill. Showers fell in the early morning, and until about 1:00 p.m. cloudiness and sunshine alternated. In the early afternoon a brisk wind sprang up from the east and grew almost at once into a hurricane varying from 70 to 120 miles per hour. This continued for the greater part of the following night and morning and was accompanied by rain. As a result, this period was marked by low temperature and high relative humidity. As a consequence undoubtedly the dendrograph curve continues to rise as late as 8:00 a.m. Thursday morning, drops until 4:00 p.m., and then continues steadily to a high apex at approximately 9:30 a.m. Friday morning. The following afternoon being very windy and dry causes a markedly precipitous drop in the curve of 19 mm. The course of the curve thereafter is considerably lower than in the first half of the week, but nonetheless shows wide diurnal variations which correspond closely with the type of weather for these three days.

Tree 3

This tree is the exact antithesis of number 2, described above, in relation to its immediate environment. It stands buried in the dense jungle of compartment 5, block III, and is exposed only partly overhead to the direct action of weather changes. It is surrounded on all sides for great distances by dense saplings, underbrush, palms, lianas, and large overtopping trees. Its crown is lower than those of the large surrounding trees, and it is thus protected from direct wind. Figure 4 shows the lower part of this tree in relation to its surroundings.

The tree itself is a small one, with a girth of only 1 foot 11¾ inches at 2 feet 6 inches above the ground, a straight smooth bole, and a small narrow crown. Very little direct sunlight strikes the bole and the ground in this region, and for this reason the rich humus soil seems damp most of the time in the rainy season. At this point the surface soil extends to a depth of approximately 2½ feet, and underneath lies a deep hard pan of marl.

The diametral changes in this tree, as shown by the dendrograph curve in text figure 5 for the week ending December 6, are quite striking in comparison with those of the others, and seem to be closely correlated with the environmental conditions described above. Temperature and relative humidity records at the base of this tree are not available for this week, but we are showing those of the same period made at tree A, about 100 yards to the east. This tree, as noted before, stands in a fairly exposed position, so that its thermo- and hygrograph records will naturally show greater variations than would occur at tree 3. These curves, nonetheless, show that this week

has a high rainfall and relative humidity and low temperature which correspond closely to the type of weather notes for this period. The temperature ranged for the most part between 70° and 85°F. , reaching the highest point in the early afternoon and often sinking below 70° in the early morning. The relative humidity drops below 80 per cent only twice and from Wednesday



Fig. 4. The base of tree 3 in relation to the surrounding jungle.

afternoon until Saturday forenoon remains almost constantly 100 per cent. Corresponding to the general weather and environmental conditions, the dendrograph curve of tree 3 for this week shows scarcely any perceptible variation. It is almost a straight line with the exception of a slight drop on Thursday and Friday afternoons and a momentary rise during Saturday and Sunday forenoons. The small drop on Tuesday morning is due to tapping and will be discussed more in detail later. The diametral changes of this tree for several weeks following were very similar to those shown in figure 5 and corresponded quite closely to the weather conditions for that period.

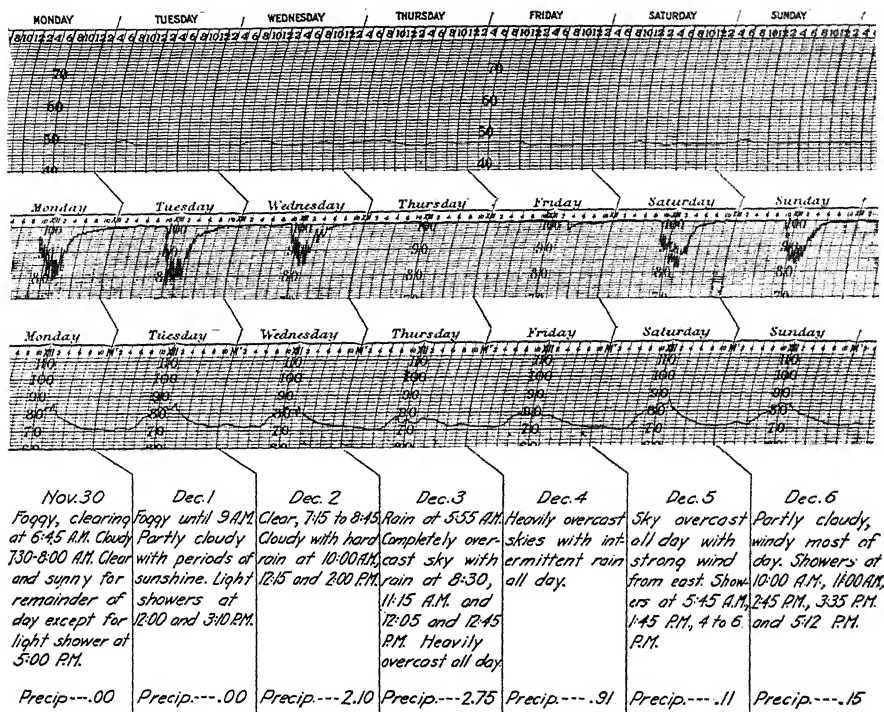


Fig. 5. The weather notes and dendro-, hygro-, and thermograph records of tree 3 for week ending December 6, 1931.

MOST FAVORABLE TIME FOR TAPPING

In relation to the most favorable time for tapping, the dendrograph records show, as noted before, that the sapodilla tree under normal weather conditions usually reaches its greatest diameter at approximately six o'clock in the morning. This maximum is generally regarded by dendrologists and physiologists as indicative of a high turgidity of the cells in the stem. On this assumption the latex vessels and latex in the cortex are also under a maximum pressure, and trees tapped at this time of the day should accordingly give a higher yield than otherwise. Thus, as far as internal conditions of the stem are concerned, approximately 6:00 a.m. in the chicle season appears to be the most favorable time to tap. However, in view of the fact that from a quarter to half an hour or more is required for tapping and one to two hours for the trees to drain, other external factors soon enter the situation, which undoubtedly influence the yield to some extent. By six or seven o'clock, depending on the season of the year, the sun is generally high enough to become effective, and usually at about 8:00 a.m. a breeze begins to blow from the east and south. This causes, as is well shown in the various thermo- and

hygrograph records presented, a precipitous drop in relative humidity between 6:00 and 9:00 a.m. and a rapid ascent in temperature. As a result there follow not only an increase in the rate of transpiration from the crown and a consequent loss in turgidity, but also a tendency for the thick, gummy latex to coagulate in the cuts and thus plug up the minute severed latex vessels. As a result the yield of latex is undoubtedly somewhat less than it would be under more constant and ideal external conditions.

This effect of different and changing external conditions on the yield of latex is well demonstrated in a series of experiments which have been running since the fall of 1927. In studying the rate of healing and recovery and conservation from limited tapping, a hundred trees have been bled in alternate years on only $\frac{1}{3}$ of the entire hole. Eight of these trees are located in the clearing around camp, and are thus openly exposed on all sides to all changes in weather. In 1927 these trees were tapped shortly after sunrise, when a brisk breeze was blowing from the northeast across the open lagoon. In 1929 they were tapped between 7:00 and 8:30 in the evening with the aid of carbon head lamps under a heavily overcast sky, low temperature, and high humidity conditions; in 1931 they were tapped in the late afternoon while the sky was heavily overcast and the atmosphere quite damp.

The results are strikingly apparent and significant, as is shown in table 1. As the sun and wind struck the trees in 1927, the latex became quite thick

TABLE 1. *Yield in cc. of latex per sq. meter of tapped surface*

Tree	1927	1929	1931
A	104.3	197.8	285.6
B	2.9	70.9	68.3
D	30.2	100.0	
E	14.9	191.5	
F	68.7	158.3	110.8
G	8.5	66.4	72.8
H	16.1	107.3	
I	52.2	188.2	124.6
Average	37.56	135.00	132.42

in the cuts, and the increase in rate of transpiration and loss of water from the crown apparently cut down the turgor of the tree to such an extent that flow ceased after a short while. As a result these trees have an average yield of only 37.56 cc. for that year. In the case of trees B and G the latex coagulated so soon that scarcely any could be collected. In 1929, when the trees were tapped in the evening under ideal conditions, the yield from a second identical $\frac{1}{3}$ panel is more than three times as great. The final third panel tapped under conditions almost similar to those of 1929 yields only slightly less. These marked differences in the three successive yields cannot be due to the stimulus of wounding, since extensive experiments of another nature along this line have failed to reveal any wound stimulus in *Achras zapota*. Successive $\frac{1}{3}$ panels tapped on the same day under similar

conditions do, of course, show some variations, but they are not at all of the same magnitude as those shown in the table above.

It is apparent from these results, as well as from the meteorological and dendrological data presented, that the most favorable times for tapping are wet, still, overcast days and during the night. The former time, however, is obviously too irregular and uncertain to be of any great significance in commercial tapping. In the late afternoons and early evenings of the tapping season the relative humidity begins to rise and usually reaches a hundred per cent by nine or ten o'clock, where it remains until about six o'clock of the following morning. In the same period the temperature gradually declines and reaches a minimum shortly before sunrise. Furthermore, as a general rule at Tower Hill, the wind usually dies down more or less completely in the evening, and the air is comparatively still until sunrise of the next day. External environmental conditions are thus most ideal during this period and last sufficiently long to allow ample time for tapping and draining of the latex. In view of this as well as the fact that the tree is apparently increasing in turgor and expanding during the night and thus doubtless subjecting the latex in the laticiferous vessels to pressure, it is apparent that both internal and external conditions are at an optimum that is only rarely realized during the daytime.

There are certain practical difficulties, however, which make commercial application of night tapping somewhat problematical. In the first place, it necessitates a head torch of some sort for illumination, and since chicleros are so reluctant to go to any extra expense, which to them would seem entirely unnecessary, this becomes a serious problem. In our experimental work we have found that the miner's carbide head lamp is well suited and operates at a minimum of expense. The initial outlay is fairly high, but such lamps might be supplied to the chicleros in quantity on the same basis that machetes, etc., are advanced. Secondly, the more or less fixed habits of the chiclero as to the proper time of tapping cause an added difficulty. He and his predecessors have been bleeding sapodilla trees in the early morning hours ever since chicle first came into commercial use, and it is hardly likely that he will change his habits without considerable persuasion, particularly when the reasons for so doing appear to him absurd. Furthermore, he argues that if it should rain during the night his latex will be ruined, since it is disconcerting, uncomfortable, and often impossible to collect the bags during the night.

EFFECT OF TAPPING ON DIAMETRAL CHANGES IN ACHRAS ZAPOTA

The native system of tapping *Achras zapota* that prevails at present throughout the chicle areas of Southern Mexico and Central America is a ruthless one in comparison to that employed on *Hevea brasiliensis* under cultivation, and is undoubtedly taking a heavy toll of the forest resources. It is essentially a half-spiral system which consists of successive parallel rows of

machete cuts ascending the bole obliquely. The successive oblique rows of cuts alternate from side to side and lead into the lower preceding ones, so that the latex from the separate series flows in a zig-zag channel down the tree to the point where the collecting bag is attached. By this system a large amount of bark is chipped out, and the extent of wounding is considerable, since each cut usually penetrates through the cambium at the point of tangency. It is to be expected thus that there would be a decided reaction of the tree immediately after tapping which would manifest itself in the periodic diametral changes. With this possibility in view in our dendrograph studies, we have accordingly tapped several sapodilla trees to which dendrographs were attached. The effect of tapping on the diameter of the trunk has been so consistent throughout that the data from three trees in different localities will suffice for the whole group.

Tree number 3, whose locality, surroundings, size, and appearance have been described in detail above, was tapped by the native system during the second week of our dendrographical studies. Because of the small huts over the instruments, tapping began about 8 feet above the ground and continued over the entire bole up to the lower limbs of the crown. This tree was tapped on December 1 between 7:18 and 7:26 a.m., and yielded 118 cc. The surrounding bush at this time was very wet, and approximately two hours later a hard shower occurred. The effect of tapping is scarcely perceptible in the dendrograph curve for the week ending December 10. A slight drop of 2 mm. occurs after tapping, but its range is no greater than that of the daily fluctuations. As shown in the weather notes for this week, relative humidity and rainfall are fairly high and continuous, and doubtless as a result of this and the location of tree 3, the dendrograph record is almost a continuous straight line for the entire period.

Tree no. 4 is representative of the cases in which tapping was done both below and above the dendrograph to test the comparative effects. In these experiments the dendrographs were mounted on the trunks midway between the crown and roots, as is shown in figure 6, with palm-leaf huts built over them. This was readily done by leaning trimmed saplings against the trunk and bringing them to a point about 4 feet above the instruments. A latticework was made around these with strips of bark to which the palm leaves could then be fastened. Readings were made in each case from a ladder.

Tree no. 4 stands in compartment 2, block II, under fairly exposed conditions. The crown is fully exposed to sun and wind on the east and south-east, while to the north and west immediately behind is a dense growth of tall Santa Maria and Silly Young trees. The tree is of the "white" variety 3 feet 1 inch in girth, with a straight bole, thick soft bark, and a perfect crown. The dendrograph in this case was placed 8 feet 8 inches above the ground and 9 feet 1 inch below the crown.

The dendro-, hygro-, and thermograph records of this tree as well as the weather notes for the week ending January 3, 1932, are shown in figure 7.

Weather conditions are fairly regular for this week, varying from cloudiness to sunshine and light wind with no rain except very brief showers on Sunday and Monday. The hygro- and thermograph curves are strikingly uniform from day to day with the exception of Sunday morning, when light showers fell. Tree no. 4 was tapped below the dendrograph with a gauge between 4:45



Fig. 6. The palm hut covering of the dendrograph midway between the base and crown.

and 5:05 p.m. on Thursday, December 31, and then yielded 103.3 cc. of latex. No perceptible drop in the dendrograph curve occurred, but this is partly due to the fact that the tree had already reached the daily minimum at the time of tapping. During the following evening, however, the curve failed to reach the maximum of the previous night, and on the next day attained the minimum of the entire week.

An interim of almost two weeks was allowed before the upper half of the bole was tapped. Tapping occurred on January 13 between 4:30 and 5:10 p.m. and yielded 203.0 cc. of latex. A slight drop in the dendrograph curve is perceptible immediately afterward, but it rose a short time later. The

dendro-, hygro-, and thermograph records for the week ending January 17 are shown in figure 8. The weather for this week was quite dry and windy with varying cloudiness from day to day. Tuesday and Wednesday mornings immediately after midnight and during the middle of the day were particularly windy, and as a result the hygrograph curve drops considerably for these periods. The last two days were rainy in addition to windy.

The course of the dendrograph curve follows these weather conditions fairly closely. During the early part of the week it shows a wide variation and then gradually tapers off and smooths out in the latter part. The effect

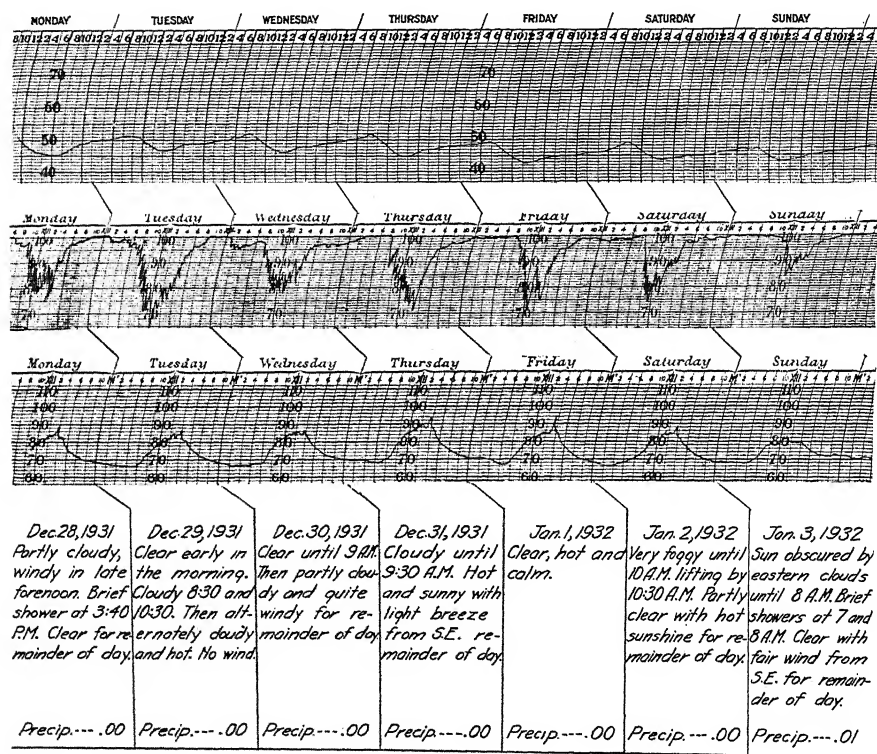


Fig. 7. The weather notes and dendro-, hygro-, and thermograph records of tree 4 for week ending January 3, 1932.

of tapping the upper half of the bole is hardly more perceptible in the curve than that produced by bleeding the lower part. Tapping, as noted before, was done in the late afternoon, when the tree had reached its minimum diameter, and this may doubtless account in part for the lack of a perceptible drop. The minimum for Wednesday, January 13, however, is not as low as on the preceding day; but this is apparently due to the fact that Tuesday was clear and very windy, while Wednesday was cloudy and a brief shower fell at 3:00

p.m. It is to be noted particularly that the curve after tapping is more even and is strikingly similar in this respect to the one of the previous week. It is true that the weather changes toward the two week-ends are in general similar, but whether this alone accounts for the more even curve is not entirely certain. It is possible that the effect of tapping is to reduce the subsequent diametral changes for a number of days.

The data from these three trees indicate that the immediate effect of tapping on diametral changes in the sapodilla tree is not as marked as might be anticipated. Tapping is generally confined almost entirely to the bark,

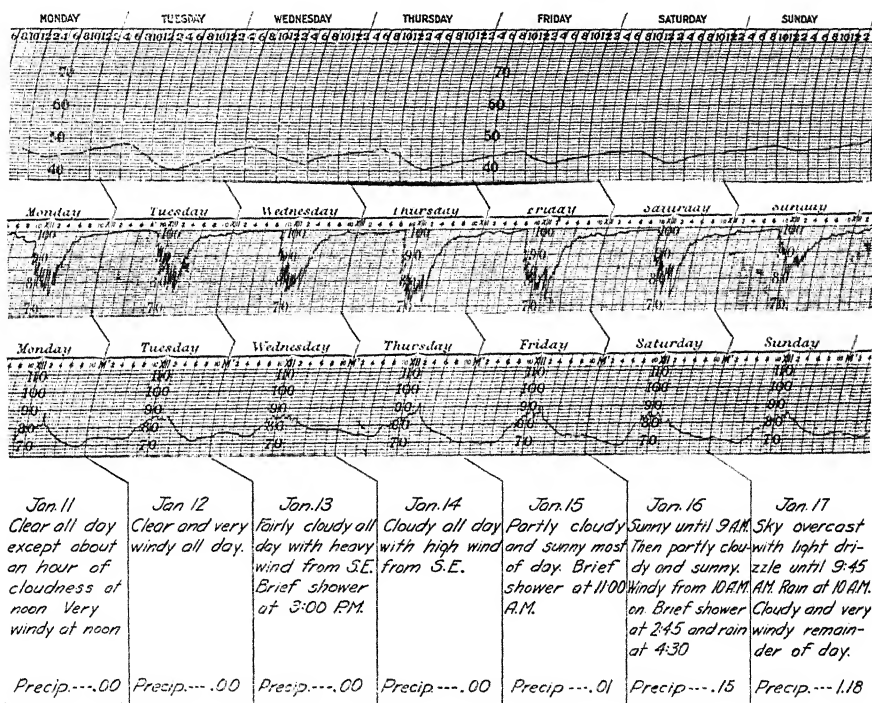


Fig. 8. The weather notes and dendro-, hygro-, and thermograph records of tree 4 for week ending January 17, 1932.

with injury to the cambium and young xylem cells only at points of tangency of the machete cuts; and in view of Haasis' (1932) observations on the Monterey pine that the bark has little influence on the daily diametral changes, this might be assumed to be partly the reason. Haasis' data, however, are hardly applicable to sapodilla trees which are being bled, since in tapping several hundred cc. of latex are drained from the bark in the course of half an hour or more, which doubtless lessens the turgor of the cortex to a certain degree.

It is, none the less, obvious from the graphs presented that the diametral

changes caused by dry, hot, and windy as well as calm, cloudy, and rainy days are far greater than those resulting from tapping. This does not seem extraordinary when the effect of weather conditions on the rate and total daily amount of transpiration from the crown of a large tree is considered. As has been shown in the meteorological notes for the various weeks, weather conditions in northern British Honduras with the exception possibly of temperature are quite variable in the rainy season during the 24-hour period. The nights are usually still and damp, while the day may vary from clear, dry, and windy to calm, cloudy, and rainy. Even the temperature may change as much as 24°F. in the course of a day. Weather conditions are therefore optimum for extreme ranges of transpiration. Accordingly, if the estimates and determinations of Von Höhnelt, Haberlandt (1892), Pfeffer (1899), Kusano (1901), Bergen (1904), Copeland (1906), Briggs and Shantz (1915, 1916), Miller and Coffman (1918), Weaver and Morgensen (1919), Sayre (1919), Burgerstein (1920, 1925), Blaydes (1928), Miller (1931), and a host of other investigators are at all applicable to tropical regions and to the sapodilla tree and accurate only to a certain degree, it is at once apparent that the amount of latex extracted in tapping is small compared with the water lost by transpiration on a hot, dry, windy day. Von Höhnelt's estimate that a birch tree with approximately 200,000 leaves standing in the open would lose 400 liters of water on a hot, dry day is doubtless somewhat inaccurate in the light of more recent and exact determinations, but it none the less indicates the rapidity with which water may be lost from the crown of a large tree. Relatively few data on transpiration rates are available for plants growing in tropical regions, and until further is known it is doubtless premature to attempt very close comparisons with the rates in temperate zones. Haberlandt (1892, 1898, 1899) claims from his studies at Buitenzorg and Grazer that transpiration in the warm humid tropical regions is two to three times less than in Europe. His data and interpretations have been vigorously attacked and refuted by Stahl (1894), Giltay (1897, 1898, 1900), Burgerstein (1897), Stenström (1895), Wright (1905), Detmer (1907), Holtermann (1902), Miyoshi (1910), and Faber (1913), who maintain that it may be as much as or even more under similar conditions than in temperate regions. The data of Giltay on *Helianthus annuus* are particularly significant, since he measured the transpiration of this species both in Buitenzorg and Wageningen, Holland, and found that the rates were almost identical. Shreve (1914), on the other hand, working on woody plants in the rain forests of Cinchona, Jamaica, found that as a result of high humidity, diffuse light, and low temperatures, the rates and total amounts of transpiration are very low and if converted to relative transpiration would be approximately the same as in xerophytic desert plants of Arizona. Holtermann (1902) and Faber (1913) maintain that transpiration in the tropics as compared with that in temperate zones is purely a relative phenomenon depending on the species of plants and the prevailing type of weather. It is well known that different species vary greatly under iden-

tical conditions, and for this reason comparative data such as cited above are doubtless of little value when dealing with a particular species.

The leaves of *Achras zapota* are quite leathery, elongate to lanceolate in shape with smooth margins, and vary usually from 24×6 to 10×4 cm. in length and breadth according to their age and position on the tree. The upper and lower surfaces are smooth and shiny with no epidermal hairs. In cross section the lower and upper epidermal layers consist of two rows of cells, the cells of the outer row of the upper layer being somewhat larger and more heavily cutinized than those of the lower. The palisade layer is usually made up of a single fairly compact row of elongated cells, but it may vary considerably in this respect in relation to environment. Often a second row of palisade cells may be present. The larger portion of the leaf is made up of mesophyll which is fairly compact immediately beneath the palisade layer, but which becomes quite loose and spongy toward the lower epidermis. Stomata are very numerous, but occur only on the under side and are not sunken or depressed. The structure of the sapodilla leaf is thus not of the markedly xerophytic type, but its leathery texture and distribution of stomata only on the underside doubtless retard transpiration.

SEASONAL SHRINKAGE IN THE SAPODILLA TREE

It has been repeatedly shown by MacDougal for many trees and more recently by Haasis (1932) for the Monterey pine that they undergo diametral changes in relation to wet and dry seasons at Carmel, California. We have found such changes to be even more marked in the sapodilla tree in British Honduras. In this country, as has been noted before, the rainy season usually begins in June and extends to the following January, with the month of November fairly dry and July, August, and October very wet. During this period light showers are likely to occur every day. In the dry season from January to June scarcely any rain falls, and the ground becomes very dry and cracked. The two seasons are thus markedly different as far as rainfall, humidity, and soil water are concerned.

Sapodilla trees whose diurnal fluctuations have been studied throughout the year show a marked difference in diameter during these seasons. Tree A, which has previously been described in detail as to location, size, and relation to the surrounding jungle, may be taken as a representative. Its daily expansion and contraction for the first week ending August 9, 1931, have already been shown in figure 2. The dendrograph curve begins at 34 mm. and ends at 50 mm., thus showing an increase of 16 mm. In view of the fact that in this part of British Honduras the available water in the soil is almost entirely dependent on periodic rainfall, we have kept daily records of the precipitation throughout the year and are thus able to correlate this factor with the diametral changes in the tree trunk. Thus, a month previous to the week ending August 9, 10.25 inches of rain had fallen, and as a result the soil doubtless had a

high moisture content. The increase in diameter from this time on was irregular but none the less progressive. The maximum mean diameter was in December, although November was comparatively dry. As is shown in figure 9 for the week ending December 21, the dendrograph curve is much higher than in August, and thus fluctuates between 51 and 61 mm. Relative humidity for this week varies from 76 per cent as a minimum in the early afternoon to 100 per cent at night. During this month the nights are comparatively long, with an early sunset and late sunrise, and largely as a result relative

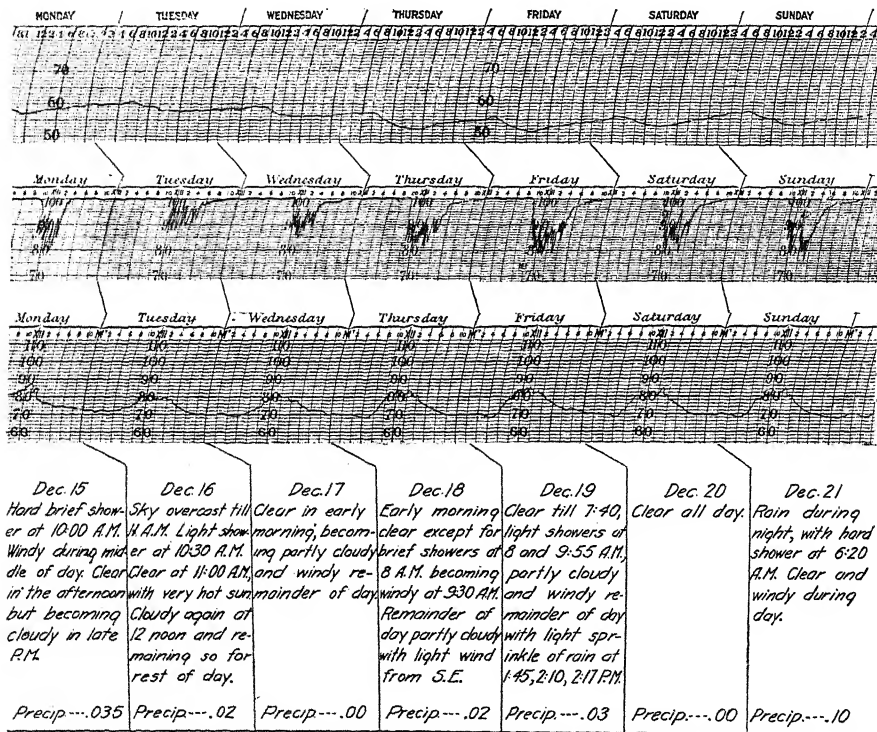


Fig. 9. The weather notes and dendro-, hygro-, and thermograph records of tree A for week ending December 21, 1931.

humidity usually remains constantly 100 per cent for more than 12 hours, as is shown in the hygrograph record of figure 9. The rainfall for a month preceding this week was 12.41 inches, and a total of 50.04 inches since August 9. At this time of the year the swamps, savannahs, pans, streams, and other low areas are usually filled with water, and the soil is generally well saturated. November and December are furthermore quite uniformly cool with the temperature varying from about 64° in the early morning to 86° F. in the afternoon.

The shorter daylight and long nights together with the low temperature

at this season doubtless reduce transpiration considerably, while the saturated condition of the soil maintains a constant water supply to the roots. As a consequence, the water balance is probably at the optimum. These environmental factors doubtless account largely for the maximum diameter of the sapodilla tree during this period.

From this time on the trunk decreases steadily in diameter until it reaches a minimum in April at the end of the dry season, as is shown in figure 10 for

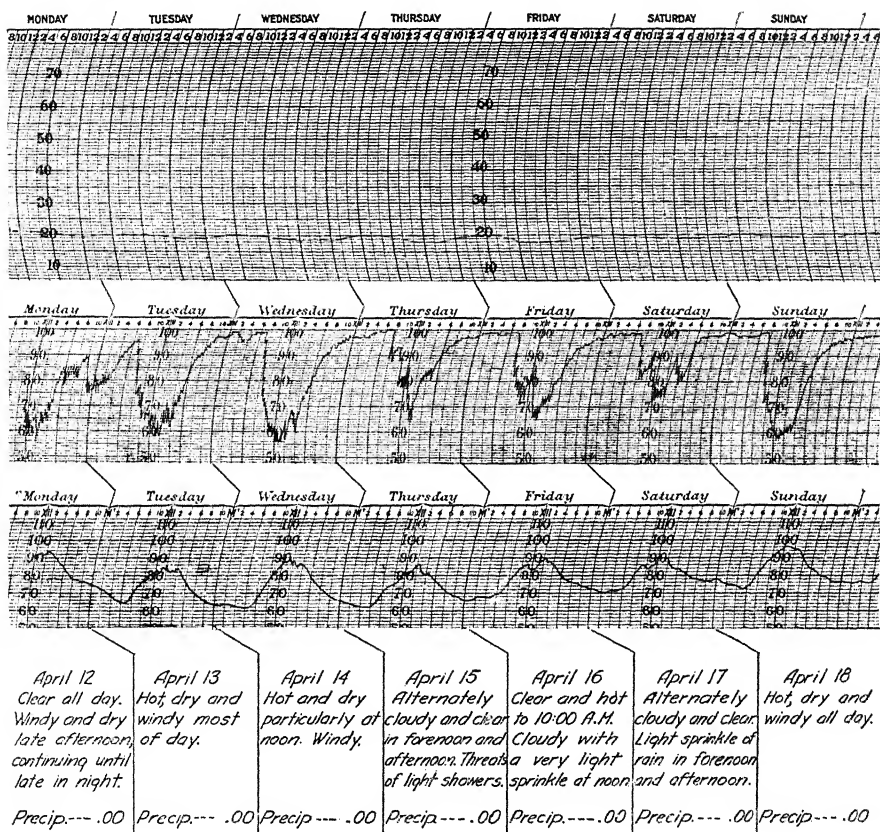


Fig. 10. The weather notes and dendro-, hygro-, and thermograph records of tree A for week ending April 18, 1931.

the week ending April 18. The dendrograph curve has dropped perceptibly since December and fluctuates during this week between 18 and 22 mm., showing thus a maximum decline of 43 mm. in the meantime. The total rainfall from December 21 to April 18 was but 5.5 inches, with only 0.5 inch recorded for the month of March. By the end of March all of the swamps, streams, and savannahs in the vicinity of Tower Hill were completely dry and the ground was quite parched and cracked.

Relative humidity and temperature show a wide range of variation for the week ending April 18. Monday night and early Tuesday morning are noteworthy, since the relative humidity dropped to 75 per cent at midnight and at no time reached 100 per cent. This was undoubtedly due to a rather brisk wind which began to blow at 8:00 p.m. On Wednesday morning it dropped precipitously from 100 to 56 per cent, and for the remainder of the week attained 100 per cent only occasionally. This wide range in relative humidity is paralleled closely by the temperature. On Wednesday, for instance, the temperature rose from 60° at 6:00 a.m. to 88° at 1:00 p.m., a range of 28° within seven hours, and continued in much the same fashion for the remainder of the period. Light showers fell during the latter part of this week, but the precipitation was not sufficient to be measured.

In spite of these extreme variations in relative humidity and temperature, the trunk shows only slight diurnal fluctuations. The dendrograph curve appears only as a slightly wavy line in contrast to its course for the same tree in August. Whether or not this lack of correlation is the direct result of water deficiency in the soil and the dehydrated condition of the tree tissues is uncertain. As has been shown previously, however, the greatest diurnal fluctuations generally occur when there is an abundance of soil water and showers in addition to wind and wide temperature variations. For the week ending April 18 there were wide variations in temperature, wind velocity, and relative humidity, but no appreciable soil water. If the diurnal fluctuations are the result of relative turgidity, it is obvious that there is a greater latitude for change on days and in seasons of greater variations in moisture, soil water, wind velocity, and temperature. The dendrograph records of MacDougal (1921) and others show that the amplitude of the reversible variations is usually greater following a rain or when the dry soil at the base of a tree is irrigated.

The seasonal variation in the diameter of tree A becomes more obvious when the dendrograph records for the entire year are observed as a whole. This may be done by plotting the weekly variations as in figure 11. The points for each week in this curve were obtained by taking the mean of the starting and ending mm. marks of the dendrograph records for every seven days. This curve shows that the trunk increases gradually in diameter and reaches its peak in December. Then it contracts gradually throughout the month of January, and after this follows a precipitous drop in diameter until the middle of April, when the minimum occurs. As the rains begin to fall again in the latter part of April, May, and June, it increases steadily until August, at which time the studies were completed. The curve is somewhat irregular on certain weeks, but this is doubtless due in large part to the manner in which the mean weekly marks were obtained. It is obvious that the occurrence of heavy rains or very dry, hot, and windy weather at the beginning or end of a week would make a wide variation in the mean points, although

the remainder of the week might have been quite uniform in weather conditions.

The amplification of the dendrograph curve for tree A is approximately 8, and the tree accordingly, between August 9 and December 21, showed an increment of 3.25 mm., which is somewhat less than the average recorded by

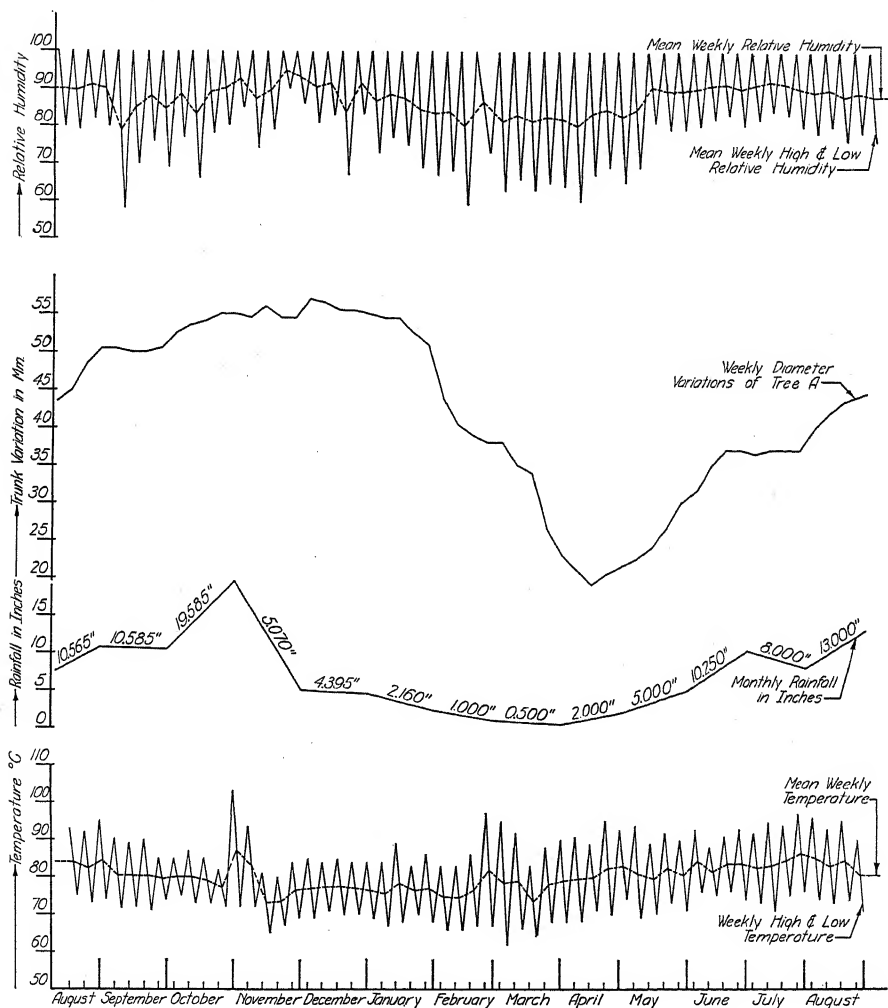


Fig. 11. The weekly high, low, and mean temperature, monthly rainfall, weekly diameter variations, and weekly high, low, and mean relative humidity of tree A.

actual measurement for the preceding six years. From December 21 to April 18, however, the diameter decreased 5.16 mm., a net loss of 1.91 mm. Dehydration or loss of water had thus offset the increment by mass and census growth.

For the sake of comparison we have also plotted in this graph the monthly rainfall, weekly high, low, and mean temperature, as well as the weekly high, low, and mean relative humidity. The dendrograph curve follows the rainfall curve fairly closely, but the two peaks do not coincide exactly. The rainfall increase during August remains uniform in September and reaches a peak in October. November and December are fairly dry in comparison, and from then on the decrease is constant. The peak of trunk diameter, on the other hand, does not occur until December, more than a whole month behind the maximum rainfall. However, it is quite probable that the decrease in rainfall during November and December was not sufficient to make much perceptible difference in the amount of soil water, since all surrounding low areas in the immediate vicinity of tree A were full of water. The soil was therefore doubtless well saturated, although the total amount of precipitation had dropped off. This together with the high relative humidity, longer nights, and fairly low temperature, as noted before, without doubt maintained an optimum water balance and high turgidity of the tree. It is very questionable, however, that the December peak in diameter is due entirely to rehydration of the cells resulting from optimum environmental factors. Enlargement due to census growth or cell division is highly probable, and must be taken into consideration.

The mean weekly temperature is fairly constant throughout the year and varies only from 71° to 86°F . There is thus no visible correlation between trunk diameter and seasonal temperature variation as has been shown by MacDougal, Korstian, and Lodewick in temperate zones. While the mean is more or less uniform, the daily temperature, on the other hand, is unusually variable. The nights as a rule are quite cool, while during the day the temperature may rise beyond 100°F . On October 15, for instance, minimum and maximum temperatures of 62° , 60° , 59° , and 58°F ., and 93° , 95° , and 98°F . were recorded during the morning and noon hours of these months. It is thus obvious that daily variations of as much as 40°F . may occur within 24 hours, which compare favorably with the extreme ranges reported in temperate zones.

As to relative humidity, there is closer correlation between its mean and the dendrograph curve than in the case of temperature. The period of highest mean relative humidity is simultaneous with the maximum rainfall and trunk expansion during the rainy season, while the lowest occurs in the dry season when the two other factors are also at a minimum. There are a few exceptional days in October, November, and December when relative humidity dropped very low, and these naturally throw the curve off of an even course. In view of the fact that relative humidity almost without exception reaches 100 per cent at night in the tropics, it is obvious that fluctuations in the mean curve will be determined largely by the daily and weekly low points, and thus show but little amplitude.

DISCUSSION

The size changes in trees, succulent plants, fruits, and leaves, shown by auxo- and dendrograph measurements, are fundamentally the result of cell activities, and as such involve directly the phenomena of growth, reproduction, and nutrition. Any analysis, therefore, of auxo- and dendrograph data must be in terms of the activity of individual and groups of cells. There are three closely correlated factors which play the dominant rôle, it seems to me, in the gradual increment of the stem during the rainy season: (1) rehydration (Haasis, 1932), (2) mass growth, and (3) census growth. The lack of concrete and conclusive evidence in each instance and the fact that they seldom occur independently, however, make it difficult to differentiate sharply and to evaluate the individual significance of these three processes, and it is thus impossible and premature to make too hard and fast distinctions. None the less, the above analysis will hold in general, I believe, and serve to clarify the discussion which follows.

Rehydration, as described by Haasis, consists primarily of the swelling up or increase in volume of more or less flaccid xylem, phloem, and other tissue cells by the imbibition of water, and as such includes also a phase of growth. This process, however, seems to me to belong in a different category from growth, and I would thus limit the term to include only the return of cells to a turgid condition as the water supply becomes abundant. Accordingly, it does not lead to an increase in dry weight, and there is no accretion to the more dense, viscid, nitrogenous cytoplasm and cell wall material or permanent changes in size, shape, or volume. Such an assumption, however, is not based on concrete evidence, and it is perhaps premature to be too dogmatic in this respect. The soil water taken in by the mature cells is certainly not distilled and contains without doubt varying amounts of salts and food substances, which must be taken into consideration.

The second factor, mass growth or auxesis (Ganong, 1908), involves the increase in size or enlargement and volume of immature and perhaps recently divided cells. Mass growth or auxesis of Ganong as commonly described and interpreted in the general literature and text-books of botany and plant physiology, by Vines (1886), Jost (1907), Barnes and Coulter (1910), MacDougal (1919, 1924), Palladin (1922), Strasburger (1923), Robbins (1928), Maximov (1930), Miller (1931), and others, may be divided into two classes: (1) increase in size and volume with dry weight increase, and (2) without dry weight increase. The latter may be extended to include such cases as sprouting potato tubers and germinating seeds where there is obviously a loss in dry weight through respiration. Auxesis without dry weight increase as generally described is primarily a matter of imbibition of water and hydration of the colloidal protoplasm (MacDougal, 1919, 1920), and involves no accretion of such material. In this respect it is like rehydration, but none the less fundamentally different. The common text-book diagrams (Sachs, 1882, p. 2;

Chodat, 1911, p. 120; Strasburger, 1923; Sharp, 1926, p. 57; Maximov, 1930, p. 285, etc.) illustrating elongation in meristematic tissues, which are extensively copied and interpreted by many as representing mass growth without dry weight increase, are, in my opinion, based more on general appearance and assumption than on specific concrete data. At least there are no conclusive data in the literature, as far as I am aware, on measurements and dry weights of cells in the formative regions as compared with those in the region of elongation on which such diagrams are based, and for this reason it is premature to say whether there has been growth with or without increase in dry weight. Mass growth with dry weight increase may or may not be accompanied by hydration, but involves primarily a greater or less increase in the denser and more viscous cytoplasm, cell wall material, and food substances. In the case of storage cotyledons and other similar organs, for instance, it is actually associated with dehydration. The protoplasm becomes progressively drier with maturity as more starch and aleurone grains, oil, etc., are stored. In the higher animal body also it has been shown (Donaldson, 1916; Hatai, 1917) that dehydration may accompany growth and advancing age.

While rehydration, as I interpret it, is similar to auxesis without dry weight increase in that it involves primarily the intake of water and no accretion of protoplasm beyond perhaps the mineral food substances in solution in the soil water, it is, none the less, fundamentally different and does not, it seems to me, belong in the same category with growth. Rehydration or increase in turgor produces size and shape changes which are temporary, while in growth the changes are permanent. The cells change in size and take definitive shape, which usually becomes more or less fixed with maturity. For these reasons, mere increase in turgor or rehydration of cells belongs more in the class of processes like plasmolysis and recovery than with growth. Rehydration and auxesis without dry weight increase, however, may follow each other very closely in the same cell and probably overlap to a certain extent. Haasis, on the other hand, as noted before, includes auxesis with rehydration and limits the term growth to census growth alone. As an influencing factor in stem increment as the rainy season begins, however, I regard rehydration as of equal rank with auxesis.

Census growth or growth with cell multiplication is the third dominant factor in stem enlargement. There is need here for a more concise name for census growth, and I am hereby proposing the term *meresis* for this process. In the course of our discussion of growth Professor R. A. Harper suggested *merosis*, but *meresis* goes better perhaps with Ganong's auxesis, and I shall hereafter use the two terms for the processes of mass and census growth. *Meresis* independently does not necessarily involve increase in size or dry weight, except perhaps the kinoplasmic material which forms the cell plates and subsequent cell boundaries, but it provides the units in which mass growth operates. It is quite obvious in uninucleated cells, however, that without cell division auxesis would shortly reach its limits because of the nucleo-cyto-

plasmic ratio. The question as to whether auxesis precedes or follows cell division in rapidly growing tissues has been much debated in the literature, and the consensus of opinion is that, in formative regions at least, the cells increase to a certain degree in size and volume before division occurs. In the final division of cells prior to differentiation in regions of elongation, however, there is little doubt that the larger part of auxesis occurs after cytokinesis. Although there are no concrete quantitative data of measurements and dry weights supporting them, the figures of cytokinesis by Strasburger (1875, 1888), Flemming (1879), Treub (1878), Chodat, Bailey (1920), Goldstein (1925), and others suggest that mass growth may go on even during cell division, and that the two processes probably overlap. The vacuolated appearance of the cytoplasm in the later stages of mitosis and cytokinesis as contrasted with its more dense condition during the prophase at least suggests that auxesis is going on simultaneously; but here again it is not certain but that the vacuolated condition may be only the result of perhaps transformation, condensation, or aggregation to form more kinoplasmic material, etc. In many plant organs *meresis* may even involve considerable decrease in size and volume. In sporangia of fungi and algae, cleavage and spore formation are accompanied by marked elimination of water, or dehydration, as has been repeatedly shown by Harper (1899, 1914), Swingle (1903), Schwarze (1922), Bold (1933), and others. In *Protosiphon*, for instance, Bold's figures indicate a tremendous decrease in size and volume during sporogenesis.

It is very doubtful, in light of what has been said above, that the progressive increment in diameter of *Achras zapota* in British Honduras through the rainy season of June to January is entirely the result of continued increase in cell turgidity or rehydration. Without doubt the full-grown xylem, phloem, pith, cambium, and cortical elements regain their maximum turgidity fairly early in the rainy season, and thus reach their limit of expansion before the stem attains its largest diameter. Auxesis and meresis doubtless come into play as soon as external and internal conditions become more favorable, as has been shown for deciduous and evergreen trees in the north temperate zone. Brown (1915) and Lodewick (1925), particularly, by histological studies have correlated dendrograph records of growth with the processes of rehydration, auxesis, and meresis in the trunk of *Pinus* and *Fraxinus*. Brown found that the phloem elements increased as much as 50 and 100 per cent in radial diameter during early spring as a result of rise in soil water before cell division began. Whether this is due merely to an increase in turgidity or mass growth is not certain, since Brown's measurements relate only to radial diameter. However, since the newly formed phloem elements usually contract considerably in the winter as a result of the low temperature, it is highly probable that a large percentage of the increase noted is due to rehydration. Census growth soon followed in the latter part of April and continued for four or five months. The initiation and rate of growth, according to Brown, are dependent on moisture, reserve food, and temperature, and

since the first two factors are at an optimum in the spring, growth was directly proportional to the prevailing temperature.

In *Fraxinus americana* Lodewick (1925) correlated the increase in diameter recorded by the dendrograph from May to October with measurement of the width of the xylem, phloem, and cambium at different time intervals, and found that the recorded growth was only 12 to 15 per cent of that computed from actual measurements. This marked discrepancy is to be partly accounted for, as Lodewick notes, by swelling of the prepared mounts during the process of desilicification. While Lodewick's measurements in relation to meresis are not specific and concrete, they indicate, none the less, that cell division is most rapid during the period when the dendrograph curve is rising.

The data of Brown, Lodewick, MacDougal, Shreve, and others relate only to trees growing in temperate regions where marked seasonal changes in temperature occur, and are thus hardly applicable to British Honduras and the tropics in general. As has been noted before and is shown in figure 11, the mean weekly temperature at Tower Hill is fairly uniform and varies only from 72° to 86°F. throughout the entire year. Hence the initiation and rate of growth are not primarily dependent on temperature. Furthermore, since *Achras zapota* is an evergreen, the food reserve should be fairly constant and sufficient for continuous growth at all seasons. However, the other essential factor, water supply, is extremely variable for long periods of time and is doubtless comparable in its effects to the marked temperature change in temperate regions. Its effect on turgidity and auxesis is evident, but no concrete data are available from tropical regions as to its influence on periodic cell division. If the seasonal rise and fall of the dendrograph curve shown in figure 11 are indicative of varying rates of cell division and auxesis, we should expect a priori on the basis of Mer's (1892) hypothesis the presence of seasonal growth rings. Such rings have so far not been found in *Achras zapota*. The sapodilla tree is an evergreen with a slow rate of growth. The xylem is fine-grained and among the hardest of Central American woods. During the progress of our tapping experiments the rate of growth of a large number of trees has been measured annually over a period of seven years. Individual trees varied more than a hundred per cent. Variations of 17.6 mm. in diameter per year were recorded. The total increment of tree A for the preceding seven years was 32.5 mm., or an average of 4.64 mm. per year.

While *Achras zapota* is an evergreen, it appears, none the less, to have more and less active periods of growth as far as the foliage is concerned. New leaves are continually being formed throughout the year, but this activity appears to be greatly accelerated in July and August and slows up from January to June. As a consequence the crown appears lighter green in color during July, August, and September. These leaves attain full size in a short time, and hence the crown seems somewhat fuller than in the dry season. The sapodilla tree thus appears to have partial foliar periodicity which corresponds generally to the wet and dry seasons. There does not, however, seem

to be any particular period when leaf fall is conspicuously evident. While our observations in this respect are limited, it appears that leaf fall is fairly uniform throughout the year, and is possibly determined more by the age of the leaves than by external environmental conditions.

Whether this partial foliar periodicity is the result of a sudden acceleration in cell division is open to question. In deciduous trees of temperate zones the leaf rudiments for the following year are already formed before the trees go into the dormant winter stage, and growth the following spring consists largely of the enlargement and unfolding of these rudiments. As a consequence there is thus a period of rapid cell division in the formation of leaf buds just prior to dormancy, which is followed in the spring by an interval of rapid cell enlargement and lessened cell division. Although there are no concrete and specific data available on this problem as far as I know, it is highly probable that the proportion of cell enlargement to cell division in leaf *anlage* in the spring is much greater than we have supposed. This is at least supported by the smaller number of division stages that are to be found in developing leaves in the spring as compared with the period before dormancy when the *anlage* are being formed.

However, no conspicuous dormant buds are developed on *Achras zapota* in British Honduras, as far as we have observed, and it is thus premature to draw comparisons. But the occurrence of periodic extremely dry and wet periods may have to a certain degree the same effect as warm and cold seasons. It is thus suggestive at least that the apparent foliar activity at the beginning of the rainy season may be as much the result of auxesis as meresis, and that the period of most rapid cell division occurred at the end of the preceding wet season.

Unfortunately, our extensive tapping program during the chicle season did not leave time for much cytological and histological work, and we are thus unable to correlate the enlargement and shrinkage shown by the dendrograph records with the processes of mass and census growth in the tree trunk and the partial foliar periodicity noted above. For this reason it is impossible to say how much of the seasonal increment is due each to auxesis and meresis respectively. However, since tree A during the six years previous to our dendrograph studies has shown an average increment of 4.64 mm. per year, it is obvious that a large part of the increase is due to census growth.

There are comparatively few concrete data in the literature relating to periodicity of growth and particularly cell division in trees growing in the tropics. The occurrence and causes leading to the formation of radial growth rings have been studied chiefly in the north temperate zone, and consequently most of our conceptions concerning vegetative periodicity of trees are based on data from such regions. We are thus inclined, as Schimper and Ursprung (1901) early pointed out, to interpret periodicity in the tropics in terms of our knowledge of its occurrence and causes in temperate zones. That there is, however, marked periodicity among evergreens in the tropics which mani-

fest itself by partial leaf fall, emergence of new leaves, and growth rings is clearly evident from the studies of Haberlandt, Holtermann (1902), Ursprung (1904), Wright (1905), Dingler (1911), and others. While most of these studies do not deal concretely with census growth, they none the less suggest indirectly that in localities where marked wet and dry seasons occur, cell division is more rapid during the former.

The studies of Hall (1890) in Uruguay and Kraus (1895) in Bombay, Buitenzorg, Garut, and Tjibodas are significant in relation to our observations, since they were made in climatic regions more similar as far as temperature, humidity, and rainfall are concerned to British Honduras than is the United States. Kraus's studies particularly were made under strictly tropical conditions. He measured a wide variety of trees at different time intervals of the day, and his results are strikingly similar to those from trees in the north temperate zone and *Achras zapota* in British Honduras. The trees usually decreased in diameter during the forenoon and early afternoon and began to increase at approximately 3:00 p.m., thus showing daily reversible variation. *Oreodoxa regia*, for instance, varied as much as 1.02 cm. over a period of seven hours. Rain, increased humidity, temperature, and wind produced marked changes in diameter in the same fashion as in the north temperate zone. Kraus, however, made only three measurements per day, and it is thus of course impossible to determine the exact time when such changes began to operate.

The shrinkage of the sapodilla from January to May is closely correlated with the weather conditions and soil moisture for this period, and probably largely due to dehydration of the tissues of the trunk, as has been suggested by Haasis for the Monterey pine. During this period, as noted before, days are longer, clear, dry, and hot, and relative humidity reaches the minimum of the entire year. Transpirational losses are thus without doubt at the maximum, and since the soil water soon becomes inadequate to compensate for these losses, water is probably withdrawn from the tissues themselves. As a consequence, progressive shrinkage occurs and reaches a maximum at the end of the dry season.

There are extensive data in the literature of plant physiology supporting the view that the seasonal decrease in stem diameter is largely due to temporary shrinkage of cells by withdrawal of water as the transpiration losses overbalance osmosis or absorption. Darwin (1893), Smith (1906), Chandler (1915), Hodgson (1917), Coit and Hodgson (1917), MacDougal (1919, 1920, 1924), Bartholomew (1926), and others have demonstrated that during periods of excessive transpiration the water supply in fruits, for instance, is heavily drawn upon to compensate for the excess loss of water through the leaves, and that under these conditions such organs undergo periodic contraction and expansion in relation to transpiration. MacDougal's and Bartholomew's auxometric measurements are particularly significant in this respect, since the course of the curves and the periods of maximum and minimum

diameter correspond very closely to and are influenced by the same environmental factors as the dendrograph records of tree stems.

As to the diurnal reversible variations in the diameter of *Achras zapota*, it is apparent from the meteorological and dendrograph data presented that they also are closely correlated with the prevailing weather conditions in British Honduras. Lowered temperature, increase in relative humidity, and rainfall are invariably followed by a perceptible rise in the dendrograph curve, while the opposite weather conditions produce the reverse effect. The data presented are thus essentially the same as those of MacDougal, Korstian, Lodewick, Pearson, and Haasis for various trees in temperate zones. The alternate expansion and shrinkage are largely due to differences in relative turgidity resulting primarily from changes in weather. Rehydration and dehydration thus play the dominant rôle, it seems to me, in the daily reversible variations. This view is supported by data relating to other plant organs as shown by Thoday (1909), Livingston and Brown (1912), and MacDougal, who found that leaf areas and diameters may decrease to a considerable extent during the day and increase in the night.

The condition of the stem during the wet and dry seasons as shown by the dendrograph records is doubtless the determining factor in the proper time of the year to bleed for chicle. The chicle season usually begins in June and continues until January, and is thus concurrent with the rainy season and maximum diameter of the sapodilla stem. During the dry season scarcely any latex can be secured. Even if tapped at night when the drying effects of sun and wind are absent, the trees yield very little latex, which is then usually thick and tenacious. This is undoubtedly associated directly with the relatively dehydrated condition of the stem.

SUMMARY

1. The trunk of *Achras zapota* in British Honduras undergoes more or less rhythmic diurnal expansion and contraction under fairly constant weather conditions. It reaches the maximum diameter between 6:00 and 7:00 a.m. and then gradually decreases to a minimum at approximately 5:00 p.m.

2. Such rhythmic diurnal changes, however, obtain only under fairly constant weather and environmental conditions. Rain, increased humidity, changes in temperature, and wind velocity may alter the rhythm considerably, particularly during the day. The season of the year and the position of the tree in the jungle, whether exposed or sheltered, likewise alter the mode and magnitude of the reversible variations.

3. Since the sapodilla stem reaches its maximum diameter and doubtless its greatest turgidity at approximately 6:00 a.m., this is probably the most favorable time for tapping as far as internal conditions are concerned. However, before tapping and drainage of the latex are completed, external factors such as increased temperature, wind, sun, and decreased relative humidity be-

come effective and doubtless influence the yield. For these reasons tapping at night seems most conducive to a maximum yield.

4. The effect of tapping on the diametral variations of the trunk, as shown in the dendrograph records, is less marked than that of hot, dry, windy weather.

5. The sapodilla stem undergoes marked seasonal variations in diameter which correspond closely with the wet and dry seasons in British Honduras. The trunk gradually reaches its maximum diameter in December at the end of the rainy period, and declines to the minimum in April at the conclusion of the dry season. As the rains start again, the stem begins to increase in diameter. Thus the daily reversible and seasonal variations are both closely correlated with weather conditions.

6. The chicle bleeding season in British Honduras runs usually from June to January and is thus concurrent with the rainy season and the period of maximum trunk diameter.

7. Rehydration, auxesis, and meresis appear to be the dominant factors in the gradual increment of the sapodilla stem during the rainy season.

8. The condition of the stem relative to turgidity and available water supply and the external environmental conditions are the determining factors in the time of the tapping seasons.

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ANATOMY OF THE EMBRYONIC LEAF¹

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The anatomy of the lamina of the mature leaf has been the subject of numerous investigations; the anatomy of the lamina of the embryonic leaf has received but scant attention. It is therefore the purpose of this paper to present data on the anatomy of the embryonic leaf of some common deciduous trees and shrubs.

OBSERVATIONS

Ash. In *Fraxinus pennsylvanica* Marshall the essential structure of the embryonic leaf is revealed by a study of an unswollen leaf bud of late March. Each leaflet (fig. 1, 2) consists of midrib and conduplicately folded wings. The wings are composed of a limiting layer of small, brick-shaped cells and a mesophyll region of cells of somewhat similar shape and rather indefinitely layered into 5-6 rows. Provascular areas are embedded at intervals in the middle rows. In figure 2 two provascular areas are recognizable. The area which is near the tip of the leaflet shows a cell of the middle mesophyll layer divided horizontally into 2 daughter cells; the other area shows a stage in which spiral protoxylem elements are present. The midrib of the leaflet is composed of a central stelar region containing primary xylem and phloem parenchyma areas, a cortical region, and a limiting epidermal layer.

Basswood. In *Tilia glabra* Ventenat the young leaf throughout its period of development from May until the following February (fig. 3-11) is composed of 5 rows of brick-shaped, densely protoplasmic parenchymatous cells of uniform size so closely packed together that no intercellular spaces are present. This regular stratified arrangement of cells is broken at intervals by provascular areas in varying degrees of development. Each of these provascular areas arises through the horizontal division of one or more contiguous cells of the middle layer of the mesophyll, thus cutting each mother cell into an upper and lower cell. Such a stage is seen between the first and second major lateral veins in figure 10. This first division is followed by other divisions in the mother cells and in adjacent mesophyll cells until very definite areas of irregularly arranged cells are gradually built up. These progressively more complex stages in the development of veins are well illustrated by the provascular areas sketched in figures 6, 4, 11 (near tip), 7, and

¹ Contribution from the Osborn Botanical Laboratory, Yale University, Seessel Fellow.

Awarded second place, Walker Prize Contest, Boston Society of Natural History 1933.

5 in the order named. Still later stages are shown in figures 9, 10, and 11. In these stages the cell divisions have been sufficient to produce the definitely thickened, swollen, vein-like vascular regions; it is in such stages of development as these that the major lateral veins over-winter. The midrib is but

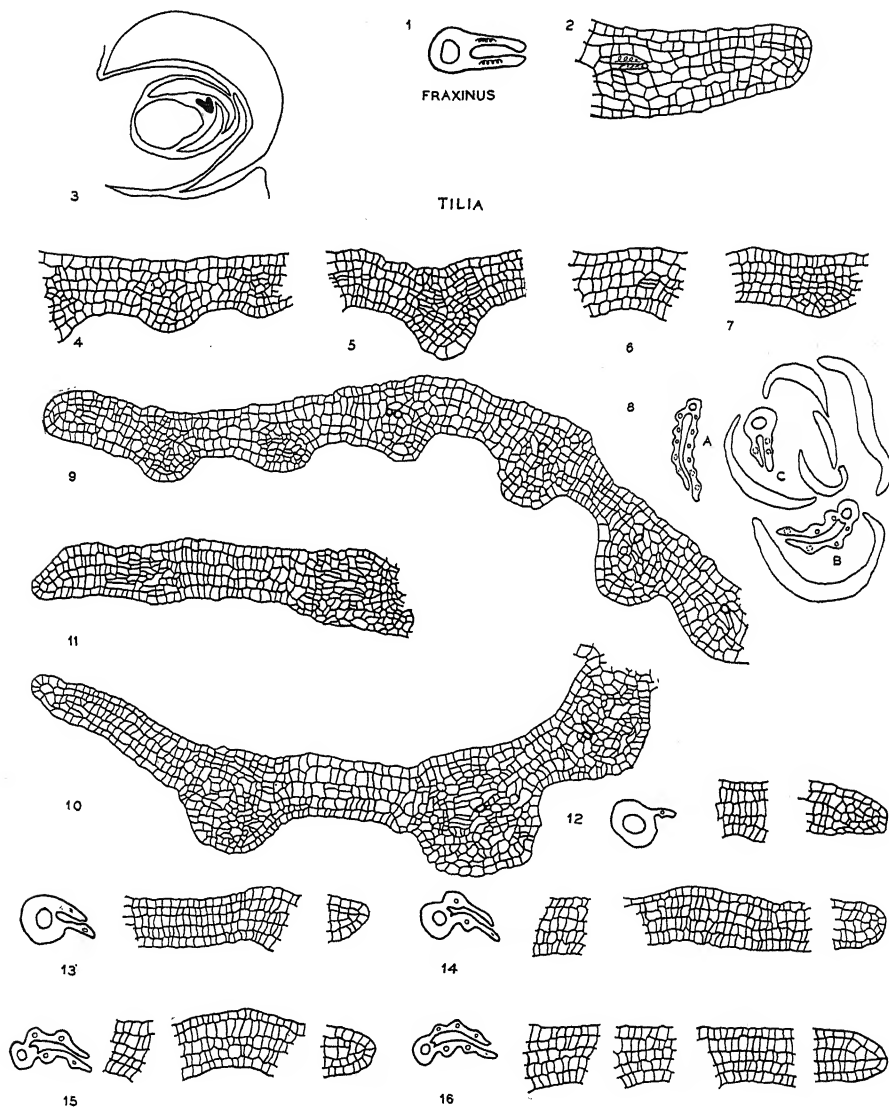


Fig. 1, 2. *Fraxinus pennsylvanica*. Cross section of leaflet, Mar. 20.

Fig. 3-16. *Tilia glabra*. Fig. 3, cross section of bud, May 4; fig. 4-7, cross sections of portion of embryonic leaf, May 4, July 6, Sept. 9, and Feb. 11; fig. 8, cross section of bud, June 22; fig. 9-11, enlargements of leaves A, B, and C of fig. 8; fig. 12-16, cross sections of leaf C at successively higher levels from base to middle of leaf.

slightly more complex in organization than are the major lateral veins. The midrib is composed of a central stelar region, of a surrounding cortical region of large, irregularly shaped cells which decrease in size toward the periphery, and of a limiting epidermal layer which is continuous with and indistinguishable from the epidermal layer of the adjacent unspecialized region. The stele of the midrib is composed of simple provascular tissue containing occasional annular or spiral protoxylem elements and groups of smaller more densely protoplasmic parenchymatous cells.

An inspection of figures 9-16 also discloses that the cells of all portions of an embryonic leaf exclusive of the provascular areas are of a uniform size. For example, if the epidermal cells of the marginal region of a leaf such as those illustrated by figures 9, 10, and 11 are compared with the epidermal cells adjacent to the midrib, no appreciable differences in size are apparent. The same is true if the mesophyll cells are compared. Likewise no differences are to be noted when these 3 leaves are compared with one another. This is of especial significance since these leaves represent three successively higher leaves from a single bud. It is equally true that no differences in cell size appear when the basal and apical portions of an individual leaf are compared, as illustrated by figures 12-16. These figures represent successive levels of a leaf from near the base to the mid-section. The small sketches at the left represent the outline of the entire leaf, and the sections at the right are enlargements of the intervein regions to show the cellular details. Had additional illustrations been included to represent the more apical portions of this leaf, these illustrations would have been exact replicas of figures 14 and 13 respectively; hence they have been omitted as unnecessary duplications.

In the preceding paragraphs it has been pointed out that the style of architecture of embryonic basswood leaves collected in May, June, July, September, October, and February remains the same. On the other hand, the total area of the leaf has increased with the advance of the season; but as the size of the individual cell remains constant, the increase in area has been accomplished solely by increase in number of cells. This increase in cell number is in part intercalary and in part marginal. Accompanying the increase in total area is a similar increase in number of provascular areas. The older provascular areas become more and more complex, and new areas arise between the older ones. Thus, in final analysis an embryonic leaf of February is an exact counterpart of the embryonic leaf of the preceding May except that it is composed of a greater number of cells.

Beech. In *Fagus grandifolia* Ehrhart leaves appear among the innermost of the stipular bud scales during the latter part of July or the first of August. The laminate portion of an embryonic leaf consists of 5 or 6 layers of cells: upper epidermis, 3 or 4 rows of potential mesophyll, and lower epidermis. The cells of all tiers are similar in size and shape, have no intercellular spaces between them, and are densely protoplasmic. This regular arrangement of tissue is interrupted at intervals by provascular areas in various stages of

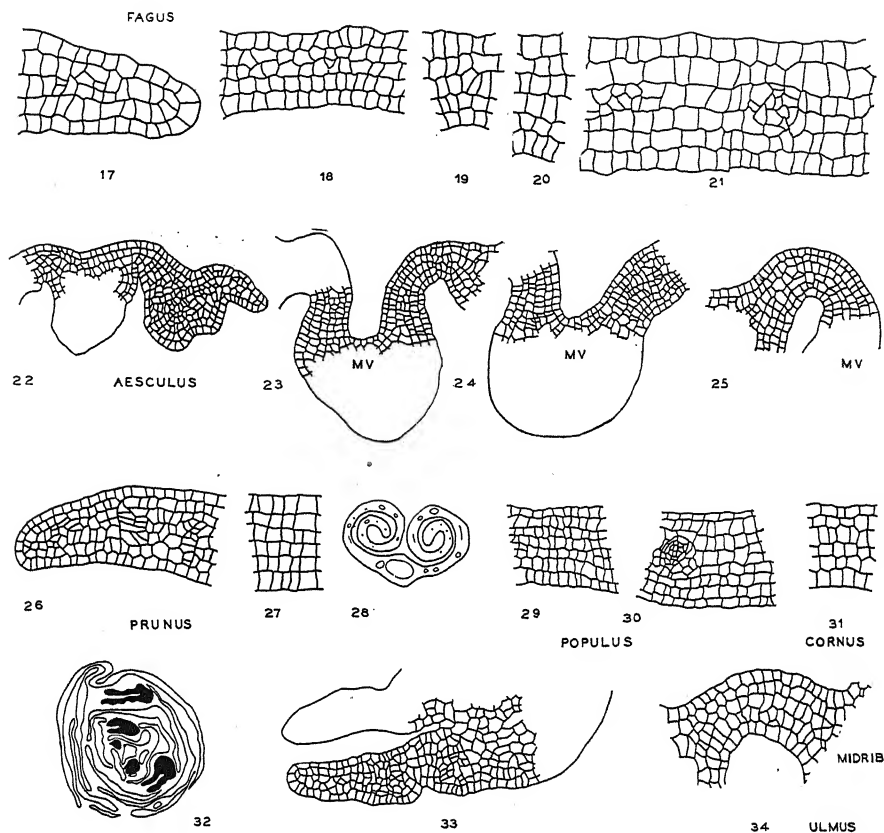


Fig. 17-21. *Fagus grandifolia*. Fig. 17, cross section of portion of embryonic leaf, Aug. 9; fig. 18-20, cross sections of portions of 3 successively higher leaves from a single bud, Sept. 8; fig. 21, cross section of portion of leaf, Feb. 11. All $\times 1200$.

Fig. 22-25. *Aesculus glabra*. July 20, Sept. 21, Dec. 15, and Feb. 11. MV, mid-vein.

Fig. 26-27. *Prunus*. Sept. 4 and Apr. 9.

Fig. 28-30. *Populus deltoides*. Cross section of leaf, Sept. 4, and 2 segments of this leaf enlarged.

Fig. 31. *Cornus florida*. Cross section leaf dissected out of bud, Apr. 9.

Fig. 32-34. *Ulmus americana*. Fig. 32-33, cross section of mid-portion of bud, Sept. 4 (leaves in solid black), and enlargement of one leaf; fig. 34, cross section of leaf, Mar. 31, from midrib toward first lateral vein.

development. Of these, the midrib is the most prominently developed. The provascular areas rise through the divisions of cells in the middle layers. These characteristics are illustrated by figures 17-21. Figure 17 illustrates a cross section of the marginal portion of a leaf of August 9; and figures 18, 19, and 20 represent sections of portions of 3 successively higher leaves from a single bud one month later, September 8. In this latter collection one of the leaves illustrated has a mesophyll consisting of 4 rows of cells; this accounts

for its greater thickness and at the same time demonstrates that leaves with a mesophyll of 4 rows of cells and leaves with a mesophyll of 3 rows of cells may both be present within a single bud. As the season progresses, the internal architecture of the leaf does not change. Thus figure 21, a leaf in February, presents an anatomical organization very similar to that of the preceding months. The leaf of February, however, has a much greater area than the one of October, September, or August. This increase is the result of an intercalation of cells between the lateral veins.

Buckeye and horsechestnut. In a recent paper on the development of the leaves and bud scales of *Aesculus hippocastanum* Linnaeus, Foster (1929) has considered the gross morphological changes occurring within a bud. A cursory investigation of this species and a detailed examination of *Aesculus glabra* Willdenow confirm and supplement these data with the following facts concerning the embryonic leaves of the buckeye. The young leaflet in July (fig. 22) consists of a midrib and conduplicately folded wings. In this stage of development the primordia of the lateral veins take up such a large proportion of the entire tissue of the wings that an interveinal region is scarcely recognizable. Soon intercalary growth is established; and as relatively few new provascular regions are added, the 5-6-tiered arrangement of the intervein region becomes more and more apparent. Thus the anatomical features of leaves of September, December, and February (fig. 23, 24, 25, respectively) are identical except for the increasing amount of intervein tissue. With the resumption of growth in February the 5-6-tiered arrangement is again obscured by the appearance of many additional provascular regions, but the general embryonic appearance of the leaf is not lost until a much later period.

Cherry. Collections of *Prunus* were made September 4, January 15, and April 9. In September (fig. 26) the leaf is composed of several rather faintly tiered rows of small, uniform, compact cells with a few vascular areas present, and all of these areas are in an early formative provascular state. The later collections reveal that no marked changes have occurred during the interim except that the leaf area has increased as a result of cell multiplication.

Cottonwood. In *Populus deltoides* Marshall the buds appear in the axils of the leaves very early in the growing season, and by early summer the young leaves have the general form and shape of the mature leaf and the internal organization of the typically embryonic leaf (fig. 28-30). The stratification of epidermis and mesophyll, however, is less clearly demarked, and the number of cells in thickness of the leaf is greater and more variable than in any of the other leaves studied. Also the xylem is more clearly discernible and differentiated earlier than in any of the other species studied. The vein shown in figure 30, for instance, is not a major lateral vein but one of the much smaller veins.

Dogwood. In *Cornus florida* Linnaeus leaves dissected out of buds in early April still present a typically embryonic structure (fig. 31). These leaves are usually composed of 6 layers of cells similar in size and shape and

closely compact. Provascular areas in various stages of differentiation are present in this homogeneous tissue.

Elm. In *Ulmus americana* Linnaeus a bud of September 4 shows the conduplicately folded leaves as interspersed among the innermost pairs of membranaceous stipular bud scales (fig. 32). The lamina of such a leaf (fig. 33) is invariably 5 cells thick, and all cells are of uniform size and closely compact with the provascular areas embedded at intervals. This 5-celled condition is a characteristic universal among elm leaves. The midrib portion of the leaf is composed of a limiting epidermal layer of cells, a cortical region of irregularly arranged cells, and a centrally embedded vascular area. This area consists of parenchymatous cells and 1 or 2 protoxylem elements. The lateral veins are entirely provascular in nature. A leaf of the following February shows no advances other than increase in area as a result of increase in cell numbers; but a leaf collected late in March (fig. 34) shows several advances over the conditions of the preceding months. The area of the leaf is greater, due in part to an increase in number of cells and in part to an increase in cell size; additional finer veins and veinlets are arising, and the midrib and lateral veins contain more protoxylem elements. On the other hand, the mesophyll of the leaf of late March still shows no evidence of differentiation into palisade and spongy parenchyma.

Hackberry. In *Celtis occidentalis* Linnaeus the leaf organization is similar to that of the elm. The laminate portion of each leaf consists of 5 rows of small compact undifferentiated cells with the major lateral veins embedded within as definite provascular areas. Leaves collected in the late autumn, in January, and as leaf emergence and expansion are taking place in mid-spring (fig. 35) differ from each other merely in total number of cells. Thus, as in the elm, the embryonic leaf is always composed of 5 rows of small, closely compact, isodiametric cells regardless of the size of the leaf or position of the bud on the tree.

Kentucky coffee tree. In *Gymnocladus dioica* (L.) K. Koch no evidence of leaf formation occurs before February. By March each leaflet of the twice pinnately compound leaves consists of provascular areas and intervein regions of usually 5 tiers of small, brick-shaped, homogeneous cells (fig. 36).

Lilac. In a young bud of *Syringa vulgaris* Linnaeus transition from outer bud scales to inner bud scales, to lower leaf to upper leaf is so gradual that no histological characteristics can be used to distinguish the one from the other during early stages of ontogeny; but as the season progresses, the outer foliar members rapidly mature into bud scales, and the inner members retain the characteristics of embryonic leaves. These inner members are composed of 5, 6, or 7 rows of small isodiametric cells, all of a uniform size with provascular areas interspersed at intervals (fig. 37).

Norway maple. In *Acer platanoides* Linnaeus the lamina of an embryonic leaf (fig. 38) consists of either 5 or 6 tiers of small, isodiametric, closely compact cells with the primary lateral veins and larger secondary veins

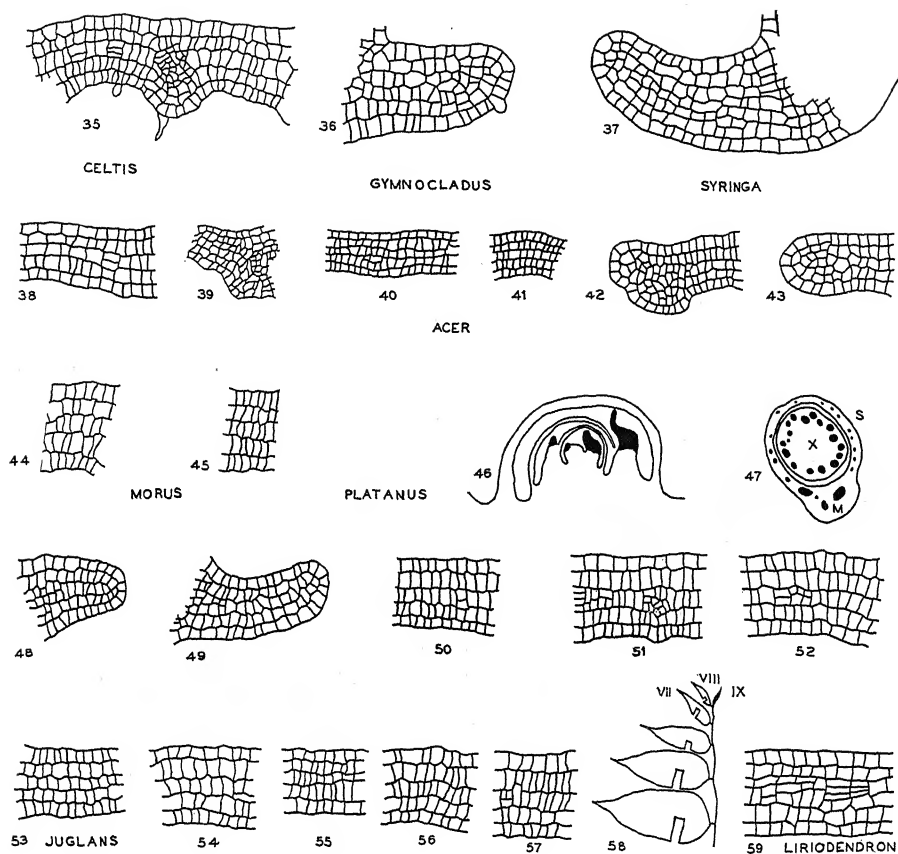


Fig. 35. *Celtis occidentalis*. Cross section of portion of leaf from expanding bud of early May.

Fig. 36. *Gymnocladus dioica*. Leaflet, Mar. 7.

Fig. 37. *Syringa vulgaris*. Feb. 20.

Fig. 38. *Acer platanoides*. Feb. 20.

Fig. 39-41. *Acer saccharum*. Aug. 24, Oct. 26, and Feb. 15.

Fig. 42-43. *Acer saccharinum*. Portion of lateral lobe, Oct. 26 and Feb. 11.

Fig. 44-45. *Morus alba*. June 16 and Sept. 4.

Fig. 46-52. *Platanus occidentalis*. Fig. 46, longitudinal section of bud, June 21, showing 4 leaves (solid black) and accompanying stipular bud scales; fig. 47, cross section in outline of basal portion of bud, June 21, showing stipular bud scale "S," petiolar portion of leaf "M," and enclosed stem tip "X"; fig. 48, tangential longitudinal section of one of the leaves shown in fig. 46; fig. 49, cross section of leaf shown in fig. 47 at higher level, enlarged; fig. 50-51, cross sections of portion of leaf, July 6; fig. 52, cross section leaf from bud, Feb. 11, showing definite establishment of palisade.

Fig. 53-58. *Juglans nigra*. Fig. 53-54, cross sections of leaflets from buds, July 20 and following Apr. 14; fig. 55-57, cross section of portions of uppermost 3 leaflets (IX, VIII, VII) of expanding leaf, May 5; fig. 58, upper portion of this leaf (1/5 natural size).

Fig. 59. *Liriodendron tulipifera*. June 16.

present as provascular areas in various degrees of differentiation. The only change occurring in the embryonic leaf after its formation in August is increase in leaf area, and this increase is caused by increase in cell numbers.

Sugar maple. In *Acer saccharum* Marshall leaves make their appearance during August. The leaf lamina (fig. 39, 40, 41) is composed of 5 or 6 rows of small, compact, isodiametric cells with no tissue differentiation save provascular areas. These provascular areas, both midrib and lateral veins, are prominently developed.

Silver maple. In *Acer saccharinum* Linnaeus the development of the leaf in its gross respects is similar to that of the sugar maple. Until midsummer the only growth of the bud is in increase in number of bud scales. At this time the young leaves begin to appear. The essential structure of the laminate part of an embryonic leaf in late October and again in February is shown by figures 42 and 43. In each case these figures illustrate the basal portion of a lateral lobe of a leaf. The leaf in both illustrations happens to be 5 cells in thickness, but in many other leaves examined the leaf was 6 cells in thickness.

Sycamore maple. In *Acer pseudoplatanus* Linnaeus (Schüepp, 1918, 1926), the organization of these leaves is similar to that of the maples described in the immediately preceding paragraphs. In one illustration the leaf is composed of 5 rows of small compact cells; in another, 6 rows.

Mulberry. In *Morus alba* Linnaeus the appearance of the embryonic leaf is illustrated by cross sections of a leaf collected on June 16 (fig. 44) and another collected September 4 (fig. 45). These leaves are 5 cells in thickness: upper epidermis, 3 rows of mesophyll, and lower epidermis. All of this embryonic tissue is composed of small, compact, isodiametric cells except the provascular areas, which show a slightly more complex tissue structure. The leaf of September 4 differs from the leaf of June 16 only in that the area of the leaf is greater as a result of increase in cell numbers. Had an illustration of a leaf as it appears the first of February been added, similar comparisons would hold.

Sycamore. In *Platanus occidentalis* Linnaeus the stipular bud scales and leaves are present by mid-June (fig. 46, 47). The laminate portion of the leaf of mid-June (fig. 48, 49) consists of several tiers of small, closely compact, cubical cells of a uniform size. By the first of July in some leaves the cells of the first row beneath the upper epidermis are slightly elongated in the direction at right angles to the surface of the leaf (fig. 50, 51); in other leaves of this same date all potential mesophyll cells are still of a uniform size. By the first of February the tendency toward elongation of the first subepidermal row is present in all leaves (fig. 52). Thus the sycamore is unique among the forms investigated in showing the tendency toward mesophyll differentiation before growth in the spring becomes apparent.

Walnut. In *Juglans nigra* Linnaeus the earliest indication of leaf formation within a bud is found in early June. These leaflets are 5 cells in thick-

ness and about 6 cells in width from margin to provascular midrib section, and all cells are similar in size and shape and closely compact. The leaf and leaflets show no further tissue differentiation during the ensuing 9 or 10 months (fig. 53-58); all leaflets are composed of 5 tiers of small compact cells essentially similar in size and with provascular areas embedded at intervals in the potential mesophyll. Figures 55-58 represent the uppermost 3 leaflets (VII-IX) of a leaf which has emerged from the bud and is over half grown. Thus the structure of the embryonic leaf of the walnut is similar to that of the other species in which the leaf is always 5 cells in thickness.

Yellow poplar. In *Liriodendron tulipifera* Linnaeus the embryonic leaf consists of small, isodiametric, compact cells. The mesophyll is not as definitely layered as in many of the preceding forms and consequently the leaf is usually roughly 6-tiered and with provascular areas exceedingly numerous (fig. 59). Occasionally leaves are 5- or 7-layered. Within a single bud the outer leaves are slightly larger in cell dimensions than the inner smaller leaves. This is probably indicative of the first stages of the differentiation and maturation of the leaf; however, the general embryonic appearance is not lost until after the leaf has burst from the bud and become extended and unfolded.

LITERATURE

A search of the literature to discover data on the anatomy and histogenesis of leaves discloses the fact that wherever pictured or described an embryonic leaf is always delineated as consisting of a few rows of compact, regularly layered cells with occasional provascular areas present. For example, Abbott (1929) includes a single photomicrograph of a leaf bud of the Tung-oil tree which shows that the intervein regions of a young leaf are composed of cells of uniform size. Artschwager (1925) portrays the basal portion of an embryonic leaf of the sugar cane as composed of a mass of compact cells with provascular areas arising through the periclinal and anticlinal divisions of cells of the middle layers of the leaf. Blaauw (1920) states that the leaf and vegetative cone of *Hyacinthus orientalis* are composed of small compact cells of uniform size. Bonnier (1900) illustrates a young leaf of *Camellia indica* as composed of simple homogeneous parenchyma with provascular areas embedded at intervals. Ertl (1932) pictures an embryonic leaf of *Xanthosoma atrovirens* as composed of 5 layers of small compact cells. Gibelli (1876) states that the young leaf of *Empetrum nigrum* consists of embryonic tissue and vein tissue. Glück (1901) states that the embryonic leaves of *Hydrocharis morsus-ranae* and *Potamogeton perfoliatus* are composed of parenchymatous, unspecialized cells with intercellular spaces absent and that the embryonic leaf in *Potamogeton* is composed of only 3 layers of cells. Goldstein (1926) describes the embryonic leaf of tobacco as composed of 6 rows of compact homogeneous tissue. Gravis (1899) states that the young leaf of *Tradescantia virginica* consists of 5 layers of small, compact cells of uniform size with provascular areas embedded at intervals. Greensill (1902)

shows that in an "immature" leaf of *Coprosma baueri* the vascular tissue is the only tissue which stands out as a distinct system; the remainder of the leaf is composed of cells essentially alike in appearance. Hayward (1932) describes the embryonic leaf of *Ipomoea batatas* as composed of several layers of small, compact cells of uniform size with provascular areas interrupting at intervals the regularity of arrangement. Herrig (1915), though studying primarily the earliest stages of leaf histogenesis, implies that the young leaves of *Elodea*, *Hippuris*, and *Galium* are composed of a few rows of compact tissue. Hirmer (1920) mentions that the young leaves of the palms are still composed of small, compact cells at the time when the division of the leaf into separate pinnae occurs. Jönsson (1878-1879, 1880) states that in *Hakea saligna*, for example, the embryonic leaf soon becomes 6 rows in depth and later by radial divisions within the rows becomes 10-12 rows in depth. Krumbholz (1925) indicates that the embryonic leaf of *Pelargonium zonale* is composed of compact unspecialized tissue. Kubin and Müller (1878) picture a cross section of an "immature" leaf of *Pistia stratiotes* as composed of a 5-layered, compact, undifferentiated tissue with provascular areas embedded in it. Langdon (1927, 1931) shows that the young embryonic leaves of *Quercus* and *Carya* are composed of small compact cells with provascular areas embedded within. Lonay (1907) describes the tissues of the young leaf of *Ornithogalum caudatum* as composed of regularly arranged rows of cells with intercellular spaces absent. Moir (1930-1931) illustrates an embryonic leaf of *Glaux maritima* which is composed of 6 rows of compact isodiametric cells. Naylor (1932) illustrates embryonic leaves of *Bryophyllum calycinum* as composed of several rows of undifferentiated cells of uniform size without intercellular spaces and of provascular elements interspersed at intervals. Rand (1922) notes that chlorotic leaves of pecan rosette are composed of undifferentiated tissue which has unmistakable characteristics of an "embryonic" condition. Schechner (1909) specifically states that the transpiring power was recorded for leaves "at the time of the beginning of tissue differentiation," "after the cuticle was thicker and the intercellular spaces incompletely built," and "after differentiation was complete." This is the only physiological paper which has come to the writer's attention which mentions the detailed internal anatomy of the leaf used. Schüepp (1917) shows that a young leaf of *Lathyrus latifolius* is composed of 6 layers of uniform cells without intercellular spaces. Skutch (1930) illustrates the leaf rudiment of banana as composed of unspecialized parenchyma cells of uniform size and provascular areas of more elongate cells. Sterckx (1899) shows that the young leaf of *Clematis vitalba* consists of 5 layers of embryonic tissue.

In the papers just mentioned the data on the anatomy of the embryonic leaf were in each instance an incidental by-product of other considerations; in the following papers the anatomy and histogenesis of the immature leaf are recognized as an integral part of the investigation. Areschoug (1897) has

recorded data on the development of the fundamental tissues of leaves of *Ribes alpinum*, *Asarum europæum*, *Iris neglecta*, *Sambucus nigra*, and *Tilia parvifolia*. In every instance the leaf is described as composed of compact undifferentiated tissue. In *Tilia* the leaves are composed of 5 rows of homogeneous cells. Thus the structure of this leaf is identical with that recorded for *Tilia glabra* in the present investigation. Billings (1905) states that the embryonic leaf of the elm is composed of "compact tissue," and his two illustrations delineate leaves having 3 rows of mesophyll. Consequently his data are in complete agreement with the author's. Collins (1918) states that in *Scaevola crassifolia* up to the time the leaf is freed from the bud the leaf is composed of a compact mass of rounded cells. Conard (1905) considers that the leaf of *Nymphaea odorata* when 3.2 cm. in diameter still retains certain attributes of wholly embryonic leaves. Famintzin (1875) states that the embryonic leaf blade of *Phaseolus multiflorus* originally consists of 6 different cell layers. In the following year (Famintzin, 1876) these data are amplified to show that the young leaves of several Leguminosae have the characteristics of strictly embryonic leaves. Flot (1900, 1903) sketches certain of the most important features of the early development of the leaf; and later (1905-1906) he elaborates the data by an extended study of the histogenesis of the leaf of *Lonicera caprifolium* supplemented by a briefer consideration of 16 other plants. A young leaf of *Lonicera* dissected out of a bud is composed of several rows of small, closely compact cells all similar in appearance and of provascular areas of irregularly arranged cells. Haberlandt (1882) describes young leaves of *Caragana frutescens* which have all cells alike, except the provascular areas embedded in the middle layers, and describes a young leaf of *Sambucus nigra* which consists of 6 rows of isodiametric cells. Lange (1927) states that the very young leaf lamina of 6 species of *Solanum* consists of 5 very regularly ordered layers. Lignier (1913) states that the mesophyll of the innermost leaves of the bud of *Cordaites ligulatus* is composed of provascular areas and homogeneous parenchymatous tissue indistinguishable from the limiting epidermal layer. Mounts (1932) describes the young grape leaf as composed of 6, rarely 7, distinct layers of small compact cells with the midvein beginning to differentiate in the second layer below the upper epidermis when the blade is about 1 mm. long; and in *Catalpa bignonioides* the developmental sequence is very similar except that the embryonic leaf is either 5 or 6 layers in thickness. Noack (1922) states that in *Pelargonium zonale* the leaf primordia early take a laminate shape consisting of 6 layers. Schwarz (1927) describes the embryonic leaf of *Plectranthus fruticosus* as consisting of 6 layers of compact cells with provascular areas arising from the middlemost layers. Yapp (1912) states that while in the bud the leaf of *Spiraea ulmaria* is composed of several layers of small, compact cells with provascular and protoxylem elements embedded in the middle layers.

DISCUSSION

An analysis of the facts concerning the anatomy and morphology of the embryonic leaf as disclosed by the present data and by the literature reveals that all embryonic leaves have the three following characteristics in common. In the first place, the embryonic leaf early assumes the shape of the mature leaf. For example, the young leaf of the basswood is oblique cordate at the base (fig. 3); and the Norway, silver, and sugar maple leaves have the three largest lobes present as definitely independent growing points by the time the leaf is 1 mm. long. Secondly, the internal make-up of all embryonic leaves is fundamentally the same. An embryonic leaf consists of a few, usually 5-8, tiers of regularly arranged, compact, densely protoplasmic, parenchymatous cells which are of a uniform size. This stratification is interrupted at intervals by provascular areas of smaller, less regularly shaped cells. Each of these provascular areas has its inception through the horizontal division of one or more contiguous cells of the middlemost mesophyll layers. Finally, the increase in area of the leaf during its initial and early stages of development is accomplished solely by increase in number of cells. Cell enlargement as a mode of increase of leaf area does not appear until after the bud has swollen in the spring. The species of plant under consideration and the stage of development of the leaf determine what portions of the leaf are the most actively meristematic; in some instances the major portion of the meristematic activity is confined to the marginal zone, in others the activity is distributed generally throughout the leaf.

As was mentioned in the preceding paragraph and as was repeatedly emphasized throughout the consideration of the embryonic leaves of the unswollen buds of the deciduous trees, all of the non-vascular portions of an embryonic leaf are composed of cells of a uniform size. This attribute of the embryonic leaf clearly reveals that the leaves as a whole are completely meristematic, and therefore no part of an embryonic leaf, not even the tip, shows signs of maturity until after the bud swells. Again, as illustrated especially by figures 4-16 and 18-20, these same characteristics demonstrate that the so-called "Laws of Zalenski" are as yet inoperative. According to Zalenski (1902), the cells of the peripheral and apical regions of a mature leaf may be smaller-celled, more xeromorphic in structure than the other regions of the leaf due to the relatively greater distance from the ultimate source of water; and for similar reasons, the upper leaves may be smaller-celled and more xeromorphic than the lower leaves. Likewise, in so far as the uniformity of cell size throughout the entire embryonic leaf contributes information to the relationship of cell size and organ size, the evidence is to the effect that cell size is not governed by organ size.

Certain of the genera studied also present data of interest toward an ultimate solution of the problem of "sun leaves" versus "shade leaves." For example, in the basswood the primary thickness of the embryonic leaf is

always 5 tiers: upper epidermis, 3 rows of mesophyll, and lower epidermis. On the other hand, Hanson (1917), in his study of the leaf as related to environment, illustrates a mature basswood leaf from the south periphery of an isolated tree as showing a clearly 4-tiered mesophyll, whereas shade leaves taken from the center of the crown of the same tree and from a forest tree show only 3 tiers of mesophyll. Again, in the sugar maple the present investigation brings out the fact that the number of rows of mesophyll present in an embryonic leaf may be either 3 or 4, whereas Hanson's illustrations delineate a sun leaf from the south periphery of an isolated tree as possessing 6 rows of mesophyll; a shade leaf from the center crown of an isolated tree, 4 rows; and a leaf from a forest tree, 4 rows and a second leaf from another forest tree, 3 rows. Consequently the pronounced sun leaf has a greater number of rows than an embryonic leaf. In a similar fashion the data for the silver maple show that a sun leaf may have more than the maximum number of 4 rows found for the embryonic leaf. Thus it is probable that the thickness of the mesophyll of the mature sun leaf may be greater than the thickness of the embryonic leaf, but that the thickness of the mature shade leaf is always the same as that of the embryonic leaf.

SUMMARY

1. Epidermis, palisade, and spongy mesophyll tissues remain undifferentiated until the bud has swollen.

2. The vascular system appears as a distinct tissue very early in the ontogeny of the leaf, and each new provascular area has its origin through the horizontal division of the cells of the middlemost tier of cells of the embryonic leaf.

3. The form and the shape of the leaf are determined early in ontogeny.

4. The growth of the leaf until the end of the winter dormant period is entirely through cell division.

5. The cell size of the embryonic leaf remains constant regardless of the organ size.

6. The embryonic leaf consists of 5-8 tiers of regularly arranged, brick-shaped, densely protoplasmic, parenchymatous cells and provascular areas of smaller, more rounded, irregularly arranged cells.

7. The thickness of the embryonic leaf of basswood, elm, hackberry, and walnut is definitely fixed as 5 tiers. In other genera the number of tiers may vary between 5 and 8 or more, but the number of tiers is constant throughout a single leaf, though not necessarily so for all leaves of a single bud.

8. The thickness of the mesophyll of the mature sun leaf may be greater than the maximum number of tiers of cells of the embryonic leaf; but the thickness of the mesophyll of a mature shade leaf probably is always predetermined in the bud.

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All illustrations are drawn with a camera lucida to an initial magnification of $\times 440$ except figures 1, 3, 8, 28, and 32, which are $\times 50$, and figures 18, 19, and 20, which are $\times 1200$ ($20 \times$ ocular 3 mm. water immersion objective). The camera lucida was bought with a grant from the A. A. A. S. and the water immersion lens with a grant from the Elizabeth Thompson Research Fund.

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OBSERVATIONS ON SAP HYDRAULICS¹

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Ever since you did me the honor of electing me to the highest office in this national Society, I have been anticipating this occasion, realizing it as an obligation and an opportunity, considering the subject of my discourse. Among my predecessors one has celebrated the occasion in blank verse, another in an opulently unblank check, another by illness and absence, others by critical surveys of the state of the world so far as our science is concerned, a few by reproof for the past and admonition for the future; and my immediate predecessor, by a remarkable summary of his life's work, informed and charmed us to a degree which I cannot hope to equal. But following afar off the example of our recent Canadian President, gifted to a rare degree with contagious enthusiasm, I shall talk about one of the things which interest me most, one which has interested all persons curious about Nature—namely, how water goes up a tree or, to borrow the apt expression of Woodhouse (1933), one of my students, *sap hydraulics*.

Almost every botanist has had something to say about this question, and therefore I am forced either to a catalogue of names or to the mention of only very few from among the many. I shall follow the latter course, spending most of my allotted time in what I choose to call reflections. In order to set my reflections on a solid foundation, however, I wish to state and to analyze the problem.

The ordinary land plant, flowering or fern-like, has a vegetative body which lives in two worlds—the soil and the air. These are indeed two worlds, different in composition, physical states, and mechanical properties. While we think we know the air, we must be quite certain that we do not know the soil. We may regard the air as a solution or mixture of oxygen and other gases in nitrogen, with which also the vapor of water is associated in larger or smaller proportions. We know the effects of higher and lower temperatures, and of relative temperatures, upon the solubilities and saturation points of water in air. But most of us give no thought to whether the air is *pure*, to what the possible impurities may be, and what effects they may have upon the physics and chemistry, the physiology, and even the psychology, of objects

¹ Address of the retiring president of the Botanical Society of America, Boston, December 29, 1933.

in the air. May I, however, defer for a time any discussion of this particular question?

Passing to the soil, I wish to point out that we speak and think of *the* soil, knowing, however, that no two soils are identical in composition, physical state, and mechanical properties. To the student of plant nutrition I leave the chemical questions as to the food materials contained in soils and soil solutions; but I call your attention to the effects of the physical states of the soluble and insoluble soil constituents upon the quantity, position, and permanence of water and of the substances dissolved in it; for these are of the utmost importance in relation to the roots as the water-absorbing organs of land plants. Those experiments of the old-fashioned laboratory of the physiology of plants (as distinct from those à la mode laboratories, miscalling themselves general physiology) which exhibit the marked adsorptive properties of certain soil constituents are still useful in suggesting many unanswered and, at present, unanswerable questions. When we compare the adsorptive and water-holding properties of rich humus with those of sand, we may well reflect upon the behavior of forest, chaparral (or scrub), and grass-land as we see them in undisturbed Nature; but when we compare these properties, we must realize the correlated phenomena, among which I wish to mention soil aeration and its relation to the formation and size of root hairs. The redwood, *Sequoia sempervirens*, is said to have no root hairs; but its old and established trees live in stands over, in, and under such a layer of humus—or shall I say of organic soil colloids?—that its drying out, that the replacement of water by air in it, is a very slow process in the laboratory and never occurs in undisturbed Nature. On the other hand, the gravels of a moraine become a huge filter when rain or melting snow goes through them, passing on its downward way the hairy roots of the trees, shrubs, and the herbaceous plants which cover its surface.

The ordinary experience of everyone who germinates seeds in wet filter paper, soil, and water for comparison, and the observation of the pedestrian who sees the hairy roots of weed seedlings on clods and in loose soil, show that the length, size, and number of root hairs on ordinary roots are directly proportioned to the amounts of available oxygen. This is the converse, to be sure, of the older statement that root hairs develop in proportion to the difficulty of getting water: for where air is in the soil, water is not, and vice versa. The fact of the matter appears, then, to be that in loose, sandy, and therefore well drained and well aerated soils, most plants develop root hairs abundantly; whereas in deep humus, in clay, in water, and in submersed mud, root hair development is weak or wanting. We may, therefore, connect the absence of root hairs on the roots of *Sequoia sempervirens*, *Nymphaea*, etc., with the lack of oxygen—with the sufficient stock of water. Whence, however, do these underground parts, often very extensive, secure the oxygen presumably required by their component living cells? In answer to this question I refer you to the familiar photosynthesis equation and to the work

of Cannon (1932), who has called attention to that term in the equation to which none of the rest of us paid any attention—namely, the oxygen liberated.

If one perform the familiar bubble-counting experiment with amputated fragments of submersed water plants—*Elodea*, *Ceratophyllum*, *Myriophyllum*, etc.—and compare the behavior of fragments and of whole plants under the same conditions, one will observe that no bubbles of oxygen leave the unwounded plants, but that their buoyancy increases with the accumulation of oxygen within them. This oxygen, in the course of unlighted hours, disappears; but though I know of no experiments designed to test this conclusion specifically, I am confident that it disappears quite as much from use in respiration as from loss by diffusion into the surrounding water. This observation strengthens the belief that photosynthetic oxygen helps to maintain the water-absorbing system of forest trees, whether their roots are in soils rich or poor in organic colloids.

I have a summer cabin in the Sierra Nevada Mountains, about 6500 feet above sea level. It is 200 or more feet above Fallen Leaf Lake, which, but for a terminal moraine, would be a bay of Lake Tahoe. Fallen Leaf Lake lies between two lateral moraines, to the eastward and westward, and between a terminal moraine to the northward and the gorge (Glen Alpine) scraped, like the mountains above, by a glacier which filled it till the present period of dryness. The surface of this region is diversified. Part of it consists of bare ledges, only in the cracks of which are there more conspicuous plants than the lichens. There are larger and smaller accumulations of humus, loam, organic soil colloids. Some of these may now be in slight depressions, may be now or may have been on the margins and bottoms of glacial lakes filled and dried by vegetation. There are forested slopes and levels broken by rocky outcrops. There are areas of shale, of the sloping surfaces of lateral moraines, and of the level surface of terminal moraines, through all of which water streams rapidly and on which forest trees lead an uncertain existence, short-lived, short-bodied, a prey to many enemies.

The Alpine vegetation of this region has been the object of my observation and reflection for twenty years, but of special intentness in the last three summers, 1931, 1932, 1933. The snow and rainfall of the winter of 1930–31 amounted to scarcely half the average or so-called normal. The snow and rainfall of the succeeding winter was about normal but so distributed that the major part of it came before the middle of January; the second half of the winter and all the spring had little precipitation. On the other hand, the snow and rainfall of 1932–33 amounted only to two-thirds of the normal, but it came after early January and was conserved by an unusually cool spring and summer. The vegetation expressed the effects of these differences in precipitation, the differences in water-conducting and water-holding powers of the materials of the land surface, the capacity of plants to adjust themselves to water intake, water lift, and water loss, according to soil and season. In the summer of 1931 even the meadows dried early,

the annuals were short-lived and stunted, the perennials, both shrubs and trees, evergreens and deciduous, reduced their spread of foliage by the most extensive summer leaf fall I have ever witnessed. Those pines and firs approximately thirty to fifty years in age suffered much more evidently than trees of upwards of a hundred years of age. The young pines and firs began the summer of 1931 with a heavy head of dark-green foliage, the older trees with a dome of foliage relatively much thinner. On the lateral moraine near my cabin the trees are all comparatively young, and growing on porous glacial gravel. They suffered much more severely than trees of the same age in soil of greater water-holding capacity. As we all know, abundance of soil-water promotes vegetative growth; but is this because of superior water-intake only? Or is it due to a favorable balance among the three stages of sap hydraulics, water intake, water lift, water loss?

That the system broke down at one or more points was evident, not only from the summer leaf fall already mentioned, but also by the number of firs and junipers which, while remaining green throughout the major part of their height, were dead from the tip of their excurrent stems downwards for at least ten feet—in many instances much more—together with the short top branches. I am not forgetful of the inroads of parasites, both plant and animal; but I think I am right in believing that the attacks of these parasites are resisted much better in seasons following abundant snow and rain than in dry summers. The struggle for existence in every mountain country is much more intense in dry than in more favorable years. The survivors of these adverse seasons become the old trees which are less hurt than those meeting severe conditions for the first time in their lives. In other words, the mortality from drought is much lower in old forests than in a young stand. Why? Among the reasons one should recognize the greater extent and depth of the root systems of old trees than of young trees, and also the accumulated litter and the greater amount of water-holding humus in old stands than in young. In mixed stands of older and younger trees the more favorable ratio of root to top in older trees aids in the repeated survival of adverse conditions by these older trees.

That I am right in believing that there is an accurate correlation among the three phases of the water system of a tree I feel is confirmed by an old pine—to cite only the one I know best. This pine is growing on a ledge, its great branches are few and widely spreading. They carry scanty brushes of needles near their tips, and spring with extraordinary balance of load and strain from a very stout tall trunk rising from a very extensive system of roots. The roots are exposed here and there as to their older parts, but penetrate to great distances and support the tree erect in a spot exposed to very severe winds. Tall, extensive, wide-spreading, but in a terrain far from nutritious or moist, this tree absorbs enough, conducts enough, to meet the needs only of a thin and repeatedly perforate dome of foliage. From year to year and according to snow and rainfall, early or late, scanty or

abundant, as I have described, this old pine, and the forest generally throughout the region, carry few or many needles, and are grey or green throughout the season until the winter's snow comes again.

Turning now from these matters of soil moisture and soil and root aeration, I should like to consider the relations of soil and air temperatures to the problem of sap hydraulics. I shall presently say more about the effects of low air temperatures. Because I come from a mild and dry climate, it may seem presumptuous to speak to you who are familiar with cold about some effects of low temperatures. We westerners are expected to talk about water relations, and of course I am following in the tradition by my subject this evening. We expect you to study frost effects, cold dormancy, and hardening. So, because I wish to say something about cold, I invite you once more to come west and with fresh eyes and minds, unaccustomed to the landscape and its problems, to observe, to reflect, and to discuss Nature as we see it in the vicinity of Stanford University.

Aesculus californica, the California buckeye, is the first tree in our region to put out its new leaves in the spring, and it is the first also to lose them, retaining them until early fall in well watered spots or dropping them completely in July or in August in drier situations. It is a small tree, growing to a height of 10-30 feet and of diffuse habit. Its leaves are large and thin; its branches and stems a delight anatomically, for they cut very readily even with an old-fashioned razor, and hand sections contain wood and bast composed of all the elements figured in the best books. This tree has the most perfect looking vascular bundles that I know, yet in stature it is unimpressive, its foliage is fugitive, its geographical distribution limited, its usefulness unimportant. In late summer its pendent fruits, pear-shaped and pear-colored, catch the eye till the winter rains have split the shells and softened the soil and made prompt germination of the large seeds possible. The leaves turn yellow, brown, and drop when, in spite of an apparently perfect stem anatomy, there is a striking water deficit in the xylem of the vascular bundles. If one make the old established India ink experiment at this time, or use a suspension of very fine starch fragments (Peirce, 1931), amputating a leafy branch under the surface of the suspension, it will be found that the particles are drawn up through very few of the wood elements, the majority of the components of the wood being filled with air. One may allow the amputated branch to stand for a time in the suspension, and thereby secure the tracing of the course of water *in bulk* through the branch, in addition to getting the injection of the suspension immediately following the amputation. If starch suspension is used, subsequent heating of the branch will fix the starch in place, and sectioning will not transfer the suspensoid from one vessel to another. Iodine will conveniently color it. Thus one sees clearly distinguished from each other, the wood elements carrying water in bulk and those containing air, respectively the hydraulic-pneumatic systems of MacDougal, Overton, and Smith (1929).

In the experiment which I have just described, "the simple precaution of preventing access of air when cutting open closed tracheal systems," to quote a recent paper of Priestley's (1932), was obviously and adequately observed; but the relative proportions of hydraulic-pneumatic systems as thus displayed at the season of maximum water-deficit and their proportions earlier and later are, however, quite different. In late September and early October, before any rain has fallen, the wood of the vascular bundles of the California buckeye fills with water. If one wound or cut off the stalk of one of the pear-shaped fruits, both surfaces of the cut will bleed profusely. If one make similar cuts at intervals down the twig, branch, and stem, one will see that the bleeding is greatest on the fruit or its stalk, and less and less as one descends the tree. No starch suspension will be taken in if the stalk or fruit is wounded below the surface of the suspension; on the contrary, the cut will bleed; but farther down a point will be reached at which there will be a water deficit and starch suspension will be drawn in through a cut made, as before, below the surface of the suspension. Between these two points there is another at which there is neither positive nor negative sap-pressure. The relative proportions, therefore, of air- (or gas-) containing wood elements (the pneumatic system) and of water-containing (the hydrostatic system) are different not only in the same region at different seasons, but in the same system of twig, branch, and stem at the same time.

To a certain degree this corresponds with the bud growth theory set forth by Priestley in the address just cited: for the large and heavy fruits of the California buckeye grow quite fast, during the dry season, accumulating water and solids at a considerable rate, just as leafy buds unfold in the spring, before the rains have ordinarily ceased and while there is still an abundance of soil moisture. The increase in water in the leafless buckeye from the middle of the dry season onward till the rains begin "must be mainly conditioned by the activity of living tissues"; but to recognize this is a very different thing from attributing the actual lifting of water through the wood to living cells. I therefore find Priestley's criticism of the work of MacDougal, Overton, and Smith as not germane or valid in this particular.

I should like at this point to ask how the water is obtained which thus develops pressure in the fruits, their stalks, and the branches which bear them; whence it comes? Granting a certain small amount of water as the product of intracellular oxidations, and a certain amount as translocated from one part of the plant to another, there is such an actual gain in weight as can be accounted for only by intake of water from the outside, by the roots from the soil. At the same time that the change from deficit to accumulation of water within this tree begins, a change takes place in its environment. Whereas the air temperatures were higher, by night as well as by day, than the soil temperatures, the soil now tends to be warmer at night than the air. That the simultaneous occurrence of these two sets of facts is not mere coincidence is indicated by experiments conducted recently by two of my students.

If one have rooted leafy willow cuttings growing in nutrient solution in jars and regulate the temperature by placing the jars in water baths, it will be found that the temperature of the nutrient solution profoundly affects the intake of water. If one keep the temperature of the nutrient solution in one jar unchanged, and lower the temperature of the solution in the other jar by rapidly cooling the bath by means of ice or of dry ice (solid carbon dioxide), it will be seen that while the willows growing in the warmer solution show no signs of wilting, even in full sunshine, the willows rooted in cold water promptly wilt. While it is well known that the rate of diffusion and of osmotic transfer is affected by rising or falling temperatures, it is obvious that in this case we have the much more pronounced reaction to a low temperature by living roots than can be accounted for by the physical-chemist on the basis of diffusion and osmotic phenomena alone.

The result above described is paralleled by the following experience. On the Campus of Stanford University are some year-old walnut seedling trees growing in undisturbed soil. While using dry ice for other purposes, the wrapped cake of dry ice was temporarily laid on the ground, and inadvertently directly over a part of the root system of one of these walnut seedlings. The other little trees presented no change in appearance, but this little tree soon began to wilt, and on the following day the leaves were dry and dead. Air temperatures were the same for all the trees; so were all other conditions except that one tree was in cold soil, the others in soil of current temperature. The air temperatures were higher than the soil temperature throughout the period of observation, a matter of days; but where the soil temperature was markedly lower than the air temperature or, as in the previous experiment, the temperature of the nutrient solution was decidedly lower than the temperature of the air, the lag in water-intake, instead of showing itself merely by a water deficit in the vascular bundles, is revealed by the wilting of the leaves.

From these experiments, which confirm our observations of Nature, we may conclude that, whenever the soil is warmer than the air, water will tend to accumulate in the bodies of vascular plants, and that, whenever the air temperatures are higher than the soil temperatures, the water balance in the plant will be lower. Whether at any one moment there will be water deficit, water pressure, or neither will depend, then, upon the temperature ratios of soil and air, in addition to all of those other factors complicating the situation. In California buckeyes growing in dry situations and leafless throughout much of the dry season we have plants in which the problem is not complicated and obscured by aging and falling foliage. These plants show their increasing water balance, even during the dry season, with the change in the air and soil temperature ratios. Other plants may be cited as exhibiting the same phenomenon. The best known of these is of course the sugar maple, bleeding when tapped in the spring, with soil warm, air frosty; with a water deficit during the summer; and filling again in the fall, before as well as after the leaves drop.

We see, therefore, that the intake of water is conditioned by the activity of living cells in the roots, and that this activity is directly related to the temperature of the surrounding medium, soil or water. I think, however, that we may go further and claim that the activity of the living cells in the leaves, and perhaps if not certainly in the trunk, controls not only the intake but the outgo and the lifting of water, that sap hydraulics is not alone a mechanical phenomenon.

But before proceeding to discuss this idea, I should like to examine the anatomy of those parts of our land plants which mainly lose water, and of those parts intervening between the absorbing and the losing parts. In the water-losing parts—leaves of varying types, cortical tissues, and fruits—we have more or less loose tissues covered for a time at least by epidermis which is interrupted only by stomata. In ordinary leaves there is no storage of water at any time, but there may be in cortical tissues and there is in the fleshy fruits. This storage in fleshy fruits may be going on at the same time that water is being rapidly lost by the adjacent leaves. Within a very short distance one may find in the fruit high sap pressure, even bleeding, in the twig much less or no sap pressure, in the leaf stalks and the leaves a water deficit.

If one turn now once more to our leafless California buckeye with its ripening fruits, we see a plant the losing surface of which is reduced to the minimum, except for the fruits, of which there is never any very large number. The structure of these fruits is much more compact, their outer surface much more waterproof, than that of leaves. Yet these are the structures which maintain those menisci from which a certain hypothesis of sap hydraulics hangs. In spite of no evident suction—on the contrary, of pronounced positive pressure—in these fruits, water storage takes place in quantity, one infers by osmosis. In the very large seeds forming in the fruits there is a heavy accumulation of starch and other foods, insoluble and soluble. In the fleshy shells there are mainly water and cellulose walls. The water and the foods for immediate nutrition and for storage come through the vascular bundles of stalk and stem. These bundles have obvious mechanical tissues to support the growing weight of fruit, and obvious conducting tissues.

The striking anatomy already mentioned of the buckeye stem exhibits xylem in which tracheids are few and ducts or vessels are many. Some ducts in each bundle are large. In none of the wood elements is the wall thick. As we all know, buckeye or horse-chestnut wood is light, soft, and loose. It is a wood the large tubes or ducts of which seem to lend themselves especially to the rapid transfer of water in masses through the lumina of the ducts. This is the water which is rapidly squeezed out when a plant bleeds through a wound, a condition which does not ordinarily prevail in any wood at the times when there is the maximum movement of water to make good what is lost by evaporation (transpiration if you choose) and the small amount which is fixed in food or storage. On the contrary, the starch suspension method,

which I have already referred to here and described in detail elsewhere (Peirce, 1931, p. 60-63), applied to a study of the course of water transport, shows that relatively few ducts or tracheids carry solid columns or wires of water, that the water is in fact in and upon the walls of the wood elements. That water which is in the walls may be thought of as fixed or bound water; but, unlike the water of crystallization, the water in a cell wall is free to move in the mass of cellulose or cellulose derivatives, though not free to leave it. Instead of twelve molecules of water being attached to one molecule of copper sulphate, as in a crystal of copper sulphate, we have in the wet cell wall an indefinite but large number of confluent water molecules among cellulose molecules, respectively a continuous phase and a discontinuous phase if you choose, or at other times both water and cellulose discontinuous. In Sponser's (1931) cellulose lattice there is ample room for enclosed water molecules, and upon the surface of the lattice adsorbed water may remain indefinitely, its outer molecules flying off into the lumen of tracheid or vessel whenever and wherever absorption exceeds use and loss, or pulled along the surface whenever and wherever the use and loss exceed absorption.

We have, then, in the xylem of the vascular bundles of land plants a freely adjustable system, varying in its proportions of thin-walled and larger-lumened vessels and thicker-walled and small-lumened tracheids and ducts. If one compare cross sections of the vascular bundles of lianes and cucurbits on the one hand with those of conifers on the other, one will realize the significance of the differences. To illustrate my point I will again describe my own observations.

One of the California native cucurbits, *Echinocystis fabacea* of the taxonomist—or big root of the layman—has a perennial fleshy root which each year sends upward one or more slender mechanically weak leafy stems with large tendrils, very sensitive to contact stimuli. The vascular bundles in their flanged stems are few and of conspicuous size, with unusually large vessels and sieve-tubes and few tracheids. Except for the masses of sclerenchyma fibers in the low flanges, most of the cells in the cross section are thin-walled. In spring, when the stem elongates very rapidly and the thin leaves spread out, the whole plant may be full of water, may bleed considerably if wounded. The underground root is a reservoir of water and of stored foods. Nevertheless, the stem is short-lived, dying before the dry season has much more than begun. With the change, often sudden and always pronounced, from the high humidity of the rainy winter season to the low humidity of the dry summer, this plant is obliged to stop growing, its ducts are empty, and the thin leaves, unable to draw water, wilt, dry, and the stem dies down to the ground. Although in the large, sometimes very large (a half barrel in size) underground and persistent part there is ample water, the carrying system is inadequate. Much more water is needed by the stem and leaves than the large thin-walled vessels carry, and the plant dies, only the subterranean portion surviving.

If, on the other hand, we examine the familiar vascular tissues of a pine, we realize the contrast—a xylem made up of tracheids, small in diameter with walls of a thickness averaging that of the diameter of the lumen. These walls are wet, containing a very considerable percentage of water at all seasons. By the starch suspension method I have demonstrated that some tracheids may carry water in mass, at least at certain times and under certain conditions, but in other tracheids I have also found the finely divided starch which I used, impacted in one side of the bordered pits but not passing through. I draw no inference from this as to whether or not the pit membrane is perforate (Bailey, 1916), for the perforations, if any, are so very small that clogging would almost inevitably occur. But I do infer that water, with very finely divided starch in suspension, moves along the walls of these unfilled tracheids. That water, with finely divided starch in suspension, moves similarly along the inner surface of the walls of small ducts and of tracheids I have seen also in that laboratory favorite, castor bean, a tree of which, several years old, is conveniently growing just outside my laboratory. But for such movement along the inner surface of xylem walls there must be a sufficient amount of water in a wet wall of sufficient thickness. In ducts, especially in large ducts with walls quite thin in proportion to the total diameter of the vessel, it may often happen, especially in dry air, in wind, in hot sunshine, that there is not enough water in the walls to maintain a film of sufficient thickness on the surface. In this case water movement will cease in a particular part of a duct. If this cessation is general, wilting, drying, and death will follow in the parts beyond.

There are certain other phenomena which I wish to couple with these observations. *First*, wilting is a common occurrence among plants with ducts; it is quite unusual in plants with xylem composed entirely of tracheids; the varying degrees of susceptibility to wilting correspond with the proportions of thin-walled ducts and thicker-walled tracheids in the vascular bundles; *second*, short-lived herbaceous annuals have very few tracheids with thick wet walls and comparatively many thin-walled ducts; longer-lived herbaceous annuals and all the perennials have tracheids with thick wet walls; the evergreens have many tracheids, the deciduous trees many ducts, the number and size of the ducts corresponding with the nature, and time of fall, of the foliage; and *third*, the height to which annuals and perennials will grow is determined, among other things, by the composition of the vascular system, specifically by the tracheids and small ducts, along the wet walls of which water will slip to meet the needs of the parts above.

These statements are bold enough to require examination, correction, or confirmation.

Under the climatic, soil, and irrigation conditions in which I cultivate the usual line of sweet peas along a trellis in my garden, my sweet peas, Spencer type, grow to a certain height, regardless of the month of fall or winter planting, but are strikingly shorter if planted in late spring. Their stems, as

we all know, are long and slender, with the vascular bundles poorly insulated in parenchymatous and epidermal tissues, and with scant space for water storage. The leaves are large and rather tender, certainly not xerophytic or well waterproofed. Their flowers and fruits are far from strongly waterproofed, and the flowers attain large or small dimensions not merely according to whether they are early or late but also according to humidity. I say humidity alone, instead of humidity and irrigation, for when my sweet pea plants are already tall, the amount of water I apply to the roots makes little impression upon the size of the blossoms if the air is dry. In other words, the vascular system becomes inadequate, it cannot carry sufficient water with sufficient speed by lift in the lumina of vessels and tracheids or by slipping along the walls of ducts and tracheids to meet the requirements, and the plant stops elongating, its flowers are no longer large and presently cease to be produced at all.

What is true of my sweet peas I believe to be no less true of other plants also. The buckeyes, as already pointed out, with their handsome and varied vascular anatomy are low trees, the conifers with their monotonous vascular anatomy attain great heights. Their only rivals, the Eucalypts of the Antipodes, also have wood close-grained which, on examination, proves to consist mainly of thick-walled elements of small diameter with large quantities of water in the walls. When, as previously pointed out, soil and air conditions make absorption and transfer of sufficient water impossible in dry seasons even in the well equipped conifers, these die at the top.

I have thus set forth an hypothesis of the course of water movement which departs from current and older conceptions in various respects. It differs distinctly from Sachs's idea that water moves mainly if not only in the walls of the vascular tissues, an idea which was shown by plugging the cavities of the xylem elements with cocoabutter or gelatin to be mistaken. I should like at once, however, to point out that both cocoabutter and gelatin not only interfere with what I have called the mass lift of water, or the columns of water of the Dixon hypothesis; but they interfere also with that thicker or thinner film of water on the inner surface of the wet-walled ducts and tracheids, delaying if not blocking that slipping along of water molecules and of masses of molecules which I have suggested. Therefore, while the experiments of Elving and others showed Sachs's idea to be wrong, interpretations of the experiments did not show more—namely, the importance of this water film lining wet walls.

The idea which I have suggested differs from my understanding of the current Dixon hypothesis in that, except as one amputates a plant under water, injecting it for a certain distance with solid water cylinders, I doubt, on the basis of my experiments with starch suspensions, whether the water cylinders in the xylem of land plants are solid at all at the time when water is moving most rapidly through the xylem and is most needed above. At these times water exists, I believe, as hollow cylinders in the small ducts and in the

tracheids, these hollow cylinders being in fact continuous with the water held in the wet walls of the wood elements.

The clever work of Bode (1923) must be spoken of in this connection. You will recall that he observed whole potted plants with more or less translucent stems: *but* the plants which he used were all of low stature (*Impatiens sultani*, *Tradescantia zebrina*, *Cucurbita pepo*, etc.) through which water can move for the short distances involved in more than one way; in some at least of which there are considerable quantities of water stored at all times; and from which, as is well known, transpiration into air of any degree of humidity is slow, and will be quite slow into air of moderate temperature and already half saturated (50 per cent humidity). When, however, the stems under his microscope did not permit him to see the ducts and tracheids otherwise, he sliced off enough of the stem to make a vascular bundle visible, and coated the cut immediately with paraffin oil. Under these conditions of low stature and short distances, of considerable stored water, of slow transpiration and fairly high humidity, Bode found continuous solid columns of water within the bundles. This he did even when the plants were partly wilted. I wish, however, to point out that, between a stem of an *Impatiens sultani* seedling laid on the stage of a microscope in a German laboratory in the showery spring and summer and an adult pine or oak of the usual height and spread, there are such differences as justify my doubt whether the water cylinders in the xylem of land plants are solid at all at the time when water is moving most rapidly through the xylem and is most needed above.

I have long been troubled to account for the absence of a hydrostatic pressure in a tall tree sufficient to burst the butt of the tree if the water in the tree were a sheaf of solid columns resting on something below and pulled up somewhere above by concave abstractions which I have never seen. There is no such hydrostatic pressure. While the weight on the butt of a tree is very great, it is the weight of the mass of wet wood, etc., not the weight of a solid column or of solid columns of water equalling the height of the tree. Instead, the great weight at the butt of a tall tree is that of the carriers and their loads. Carriers and loads are distributed all the way up from the ground to the top of the tree. The strong walls of ducts and tracheids amply support the hollow cylinders and the enclosed molecules of water, distributing the weights throughout the mass of the stem or trunk of the tree.

Perhaps you are willing to grant the existence of such enclosed and absorbed water in the xylem of our land plants, and now wish me to name and describe the actual lifting mechanism by which this water is carried from roots to leaves in our land plants. This mechanism has been variously described as living cells and as mechanics. Many experiments, to speak only of those of Strasburger and Overton, have been designed and carried out on the laboratory or garden scale to show that living cells are not involved. The chief proponents of the idea that living cells lift the water are Godlewski and Bose, the one dead, the other doubted. Thinking that, in dry ice, I had an

admirable means of testing the idea by applying killing cold to definitely limited areas of twigs or stems of plants growing otherwise undisturbed out-of-doors, I proceeded to adjust cones of waterproof paraffined paper on vertical stems and branches, fill these with water, and chill this water with dry ice. By this means I failed to produce any temperature low enough to interfere with water movement through and above the zone of 2-4 cm. in width which I chilled.

Providence, however, provided me with an experiment on the scale of hundreds of square miles, in one of those always unusual seasons which so often trouble Californians. The low temperatures of last December and January in middle California were fatal to many plants and extremely and strikingly injurious to others. That ancient castor bean tree already referred to, growing just outside my laboratory, although protected by the eaves of the adjacent stone buildings, was very heavily frosted. Everywhere the tall trees of *Eucalyptus globulus*, planted as windbreaks, wood lots, or scattered ornaments, had their leaves and their branches for considerable distances back from the tips, killed by the nightly recurring frosts. Unlike the East, this frost-killing was due to the low temperature of the air, not to frozen soil in which the water is locked. The soil was warmer than the air, and water continued to be absorbed by the root system and moved by the lower part of the vascular system in the massive parts of my castor bean and *Eucalyptus* trees. What was killed?

Following relatively mild frosts, the leaves of *Eucalyptus* look as if they had been burned, the blue-green color is replaced in spots or over the major part or the whole of the leaves by dry brown. If the leaves are only mildly frosted they do not die, though the brown spots are dead. The menisci—if there are such things—in the living cells and tissues, and the water-absorbing cellulose of the walls of living and killed cells continue to absorb water; but if the loss in the killed cells exceeds the intake, drying out results. Water loss cannot be controlled by dead cells and dead tissue. Whether this is due to increased permeability or to what, does not matter so far as we are concerned at the moment. What does matter is that the live cells in the living parts of the leaves do not lose their water and do not dry out.

Where, however, frost is severe the whole leaves may be killed and the tree then will be domed with material which will lose its own water, and all it can get besides, rapidly. But when the air temperatures are low enough, night after night, not only to kill the whole leaves but also the twigs and smaller branches, all the cells die in these less massive parts. Bulk, with the corresponding quantities of heat liberated by oxidative metabolism, carried the trunks and larger branches of *Eucalyptus* and castor bean through the severest cold of many years in middle California.

That these still living parts continued to obtain enough water is proved by the later development of buds which otherwise would have remained latent

indefinitely. The *Eucalyptus* trees, therefore, of middle California are recovering from their mutilating experience, and in the course of the next few years will have sloughed off their dead branches and covered themselves anew with foliage, normal in shape, position, and extent. But wherever the cells were killed, there water ceased to flow normally and the part dried out.

What, then, are the mechanics of water movement in the vascular tissues of land plants? In the re-examination of vascular anatomy which I have suggested to your minds, we have seen the proportions and the importance of the thicker-walled constituents of the wood and the position of water in and on their walls. I believe the quantity of water in any particular part of a vascular bundle or vascular element to be controlled by the condition in relation to water of the living cells or the living cell adjacent to this. Withdrawal of water by a transpiring leaf will effect a change in the water content of every element, living and lifeless, throughout the vascular bundles directly supplying that leaf. That the living cells within and adjacent to vascular bundles operate as suction and force pumps alternately, there never has been any evidence; but that the water balance of these cells will affect and be affected by the water balance in and on their wet walls and the wet walls of adjacent cells and elements is inevitable. Intake of water into a living cell in the xylem of a bundle, setting into endosmotic motion the water in its own cell wall, will affect the movement of water molecules in another part of the continuous mass of water which extends from tip to tip of the plant. This movement, this displacement, whether from endosmosis or exosmosis, of a few molecules at any one moment and at any one point, multiplied by millions to correspond with the numbers of living cells in the living body of a tree or other land plant, is readily conceived as adequately accounting for the movement of the very large volumes of water in the course of a year or a season. That molecule *A*, taken into a root, is immediately shot up to the top of oak or pine, does not necessarily follow; but it may well follow that the intake of water molecule *A* is due to the evaporation, the flying off into the air, of water molecule *Z* from a chlorophyll-containing mesophyll cell which abuts upon an intercellular space communicating through a stoma with the air outside. For if water molecule *Z* move out of mesophyll cell *M*, it will do so from a wet mass which is continuous from tip to tip of the tree, a wet mass parts of which are enclosed within the cytoplasmic membranes of living cells, other parts of which are enclosed within and among the cellulose molecules of cell walls, other parts of which are upon the inner surfaces of the cellulose walls of tracheids and vessels, in roots and stems and leaves. The amounts of water in different parts of a plant vary greatly, but they are all parts of the same mass. If the balance change in one part, it necessarily changes in every other part, however remote. Except as water may be split into its constituent hydrogen and oxygen, and recombined with other things, thereby ceasing to be water, none of it is cut off in the body of the plant from all the rest. Held as a constituent part of that colloidal system we call

protoplasm, held as a part of that cellulose system we call a vascular bundle, held on the inner surfaces of such vascular systems, continuous from the epidermal cells of the young roots to the epidermal cells covering the tips of stems, branches, and leaves, whatever changes the direction or speed of motion of a molecule of water at any point in the mass affects every other part of the mass, and direction and speed of molecular motion change throughout the mass.

I think we are now ready for the last detail in my picture. If the cylinders of water in vessels and tracheids are hollow, what do they contain? Obviously they contain water vapor. I need not point out that the freedom, amplitude, and speed of movement of water molecules are much greater as vapor than as liquid, and whatever diminishes the water pressure, as vapor or as liquid, in any part of the continuous mass of water in any living organism, will correspondingly draw water as vapor or as liquid in that direction. Any given molecule may in its course be drawn to others, thereby becoming part of the liquid water in or upon the wall of a tracheid or vessel; and from this it may move away into the vapor contained in the same or another vessel or tracheid. So water molecule *A*, taken into the root, may find itself alternately in a closely packed crowd on the wall of a vascular element, or part of a smaller number of dispersed molecules forming the vapor inside a hollow cylinder of water, and all the time in rapid motion in the direction of less resistance, often toward mesophyll cell *M* and out into the air in the wake of water molecule *Z* previously mentioned.

SUMMARY

If I may now summarize, I should like to say that I have set forth a general hypothesis—namely, that in living land plants, which are masses of living cells and the shells of others once living, the processes upon which the life of the plant depends are carried on by physical and chemical means, *but* that the initiation, continuance, and cessation of these physical and chemical means coincide with the life of the plant and of its constituent living cells. I would go even further and assume that this is not mere coincidence, but cause and effect. Experiment shows that the absorption of water by roots is dependent upon soil and water temperatures favorable to the living cells of the roots. Experiment, on the laboratory or garden scale, and on the grand scale of Nature, shows that the movement of water from the roots through stems and branches to the leaves is dependent upon the life of the parts intervening between roots and leaves; and that where life is, water moves; where only dead cells are, there is no ascent of sap to make good the loss by evaporation. We are all convinced, by the frosts of autumn if not otherwise, that if leaves or parts of leaves are killed, they cease to move water normally—that is to say, under control. It is true that the relation of soil and air temperatures regulates the quantities of water in the parts of a plant; the warmer the soil

in relation to the air, the more water will the stems contain. On the other hand, if the air is warmer than the soil, use and loss of water by the parts above will tend to exceed the intake by the roots and there will be only a sufficiency or even a deficit of water in the vascular tissues of stems and branches. This water, as shown in various ways including the starch suspension method, is contained in and on the walls of the cells of the vascular system as a part of the continuous mass of water in the living body of the plant. And any removal, by evaporation, transpiration, or otherwise, of any water from the mass so disturbs the whole equilibrium of the mass of water, supported everywhere by elastic cell walls, that water moves to the losing part, slipping along the surface of the elongated members of the vascular system, slipping along the inner surfaces of tracheids and ducts, as swiftly as the stream of molecules of water is lost by transpiration from the leaves, provided that transpiration is not too swift for the maintenance of a sufficient film of water on the inner surfaces of the ducts and tracheids. Within these hollow cylinders of water there is water vapor, the freedom, amplitude, and speed of movement of which are greater than those of liquid water; but the constituent molecules of which, as they move more or less swiftly up a tree, may become alternately parts of liquid, parts of vaporous water, remaining always parts of the continuous wet mass of the tree until they fly off into the air.

A study of vascular anatomy, supplemented by experiment and otherwise, shows that the maintenance of a moving or movable film of water on the inner surface of a duct or tracheid depends upon the quantity of water in reserve in the wall; the thicker the wet wall in proportion to the diameter of the member, the more certain the movement of water. The size and proportions of ducts and tracheids, among other things, determine the heights which herbs, shrubs, and trees attain, and they do this because they determine the volume and the speed of water movement. And since each duct and each tracheid, and each living cell, is a unit, although a constituent unit of the whole system of a living land plant, the amount, proportion, and speed of movement of water in one part may be quite different from the amount, proportion, and speed of movement in any other part, however close or however distant. This is my idea of *sap hydraulics*.

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MEIOSIS IN SOME SPECIES AND A HYBRID OF *PAEONIA*

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INTRODUCTION

The breeding experiments of Saunders (1933) on the genus *Paeonia*, which have now been carried on over a period of twenty years, have produced a large number of new and handsome hybrids in this well-known genus of garden flowers, and have served also to clear up many of the difficulties connected with the relationships between the various species and hybrids. In order to make clearer Saunders' results in regard to the ease or difficulty with which species can be hybridized, and the amount of sterility of the resulting hybrids, a cytological study of the peonies growing in the Saunders garden was begun by the senior author in 1929, and was interrupted by his untimely death in 1930. The work was continued by the junior author in 1932, and the original aim—that of studying the meiotic behavior of the entire series of species and hybrids, and thereby correlating cytological, genetic, and taxonomic evidence of the relationships between the species in order to determine the course and, so far as possible, the mechanism of evolution within the genus—is still in view. The present paper is an account of the results obtained by the senior author, completed by means of new observations and new preparations made by the junior author.

The observations of Langlet (1928) on the somatic chromosomes of various species and those of Sax (1932) on the meiotic chromosomes of *P. suffruticosa* have shown that the chromosomes are large and few in number, and that the chromatids are unusually distinct in the metaphases and anaphases of meiosis. Hence *Paeonia* is unusually favorable for an investigation of cytological problems of a general nature, and these will therefore be considered in the light of evidence thrown on them by an investigation of the various species and hybrids. Unfortunately, the large size of the plants and the long growing period (five to six years from seed to flower) make quantitative genetic study impossible, and therefore *Paeonia* is not favorable material for a solution of many current cyto-genetic problems.

MATERIALS AND METHODS

The preparations made by the senior author were collected in the Saunders garden during the spring of 1929. Some were fixed in Flemming's solution and others in Carnoy's fluid. Both fixatives gave quite similar results. The

material was then embedded in nitrocellulose according to Jeffrey's (1928) method, and sections were cut 10 to 20 μ thick. These were stained with Heidenhain's iron-alum haematoxylin. Those made by the junior author, on which the studies of *P. albiflora* and *P. Smouthi* are based, are smear preparations, fixed in Taylor's modification of Flemming's fluid, and stained according to Newton's iodine-gentian violet method. All drawings were made using a Bausch and Lomb microscope with binocular eyepiece attachment, a 2-mm. apochromatic objective, and 12.5 \times compensating oculars. A Zeiss Abbe camera lucida was used, the drawing paper being at bench level. The magnification obtained was about 3500, and the drawings have been reduced to about two-fifths of this size in reproduction.

MEIOSIS IN SOME DIPLOID SPECIES

As the counts of Langlet (1928) have shown, all of the species so far investigated are either diploid with $n=5$ or tetraploid with $n=10$ chromosomes. The studies of the senior author were chiefly on various species of the former type and are here presented.

P. suffruticosa Andr. (*P. Moutan* Sims.). As indicated by Rehder (1927), the familiar name for the best-known of the tree peonies must be replaced by an older one, which is, fortunately, more descriptive and quite appropriate. The plant studied is one of a horticultural strain growing in the Saunders garden.

As meiosis in this species has been carefully studied by Sax (1932), little need be added except that his results are quite paralleled by those of the senior author. The preparations were not favorable for a study of chiasma formation. Out of 100 heterotypic metaphases studied, 40 showed failure of pairing in one pair of chromosomes. The separation at the heterotypic anaphase into groups of 6 and 4 chromosomes which, as Sax suggested, might lead to genetic non-disjunction was much less common, being found in only 2 out of 100 anaphases and homoeotypic divisions studied. In a single cell of this number, a whole chromosome was found in the cytoplasm, the daughter cells which resulted from the heterotypic division having 5 and 4 chromosomes respectively.

The attachment of two chromatids at the heterotypic anaphase, shown by Sax in his figure 12, was also quite abundant in the writers' material, and is illustrated in figure 1. This connection persists throughout the interphase, and may frequently be seen during the homeotypic division (fig. 2).

In addition to these phenomena, fragmentation of a chromosome was occasionally observed at the heterotypic anaphase and during the homoeotypic divisions, 4 per cent of these stages showing such fragments. In figure 1 a fragment (*F*) consisting of a part of a single chromatid can be seen below the group of chromosomes at the left-hand end of the cell, while in figure 2 a small mass of chromatin, clearly the remains of a chromosomal fragment,

is in the cytoplasm between the two spindles of this homoeotypic division; one of the chromosomes of the left-hand spindle is apparently lacking a part of a chromatid. In both of these cases there is no doubt that a fragment, rather than a whole chromosome, has been left out, since the full number of

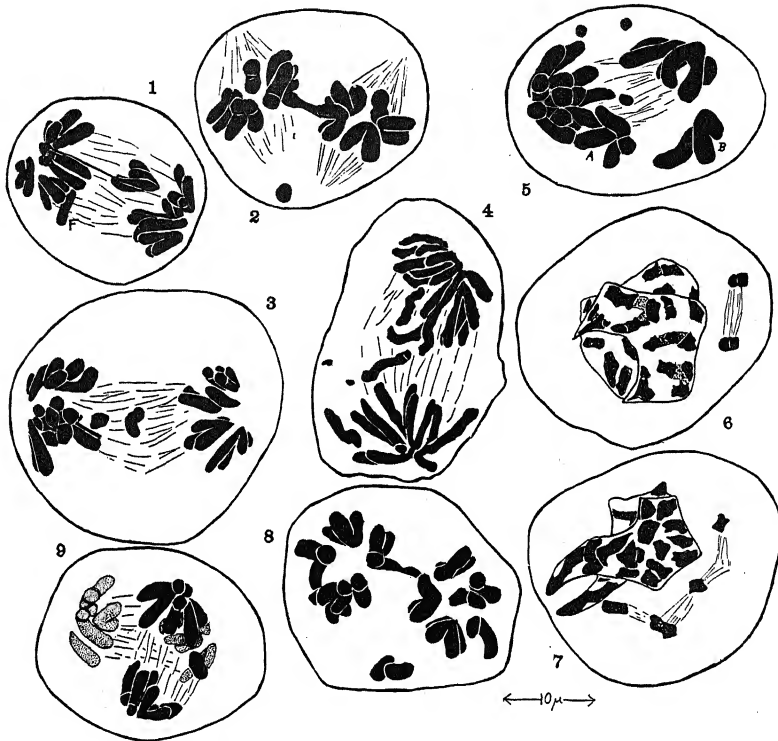


Fig. 1-9. Fig. 1. Heterotypic anaphase of *Paeonia suffruticosa*, showing chromatid fusion, and a fragment, *F*, situated in the cytoplasm below the spindle near the left-hand pole. Fig. 2. Homoeotypic metaphase of the same, showing chromatid fusion and a fragment. Fig. 3. Heterotypic anaphase of *P. Mlokosewitschi*, showing abnormal separation ("non-disjunction") and fragmentation. Fig. 4. The same, showing excessive fragmentation. Fig. 5. The same, showing extrusion of chromosomes *A* and *B*, fragments, and abnormal separation. Fig. 6. *P. Mlokosewitschi*, interkinesis, polar view, showing "mitosis" of a fragment. Fig. 7. The same, showing four fragments. Fig. 8. Homoeotypic metaphase of the same; chromatin fusion and fragments. Fig. 9. Homoeotypic anaphase, showing formation of a fragment.

five chromosomes can be counted at each pole in figure 1 and on each spindle in figure 2.

P. Mlokosewitschi Lomak. This species, native to the Caucasus mountain region, is a close relative of *P. corallina* of Europe, and is one of a group of several closely related species or subspecies, including *P. triternata*, *P. russi*, *P. corsica*, and others. These are characterized by having entire leaf-

lets, a pink color of the stem and petioles, and reflexed carpels. The experiments of Saunders, as far as they have been conducted, show that they intercross with ease and produce hybrids that are practically as fertile as the parent species. Langlet's counts have shown that they are diploid.

P. Mlokozewitschi exhibits the same abnormalities of meiosis as *P. suffruticosa*, but these are much more abundant. Out of 100 pollen mother cells at the heterotypic metaphase, 48 showed failure of pairing in one pair of chromosomes. Out of the 208 heterotypic anaphases and homoeotypic divisions studied, 19, or 9 per cent, show the separation into nuclei containing six and four chromosomes, while in 16 cells, or 8 per cent, one or more chromosomes were cast out into the cytoplasm. Of these 16, 12 showed nuclei with five and four chromosomes, while in one cell the separation was into a group of six, and one of three chromosomes. In three cells, two chromosomes were found in the cytoplasm, and in these the separation was twice into groups of five and three, and once into groups of four and four chromosomes. One can summarize, then, by saying that out of the 832 gametes which would have resulted from these 208 mother cells, 4.8 per cent would have six, 86 per cent five, 7.9 per cent four, and 0.9 per cent three chromosomes. Since in a single mother cell separation into groups of 7 and 3 chromosomes was discovered, there would be two gametes, or 0.2 per cent with seven chromosomes. Since repeated pollen tests made by Saunders have shown the pollen to be only about 80 per cent good, it is quite likely that none of the grains with an abnormal chromosome number are viable. As yet, no aneuploid individuals of this or any other species of *Paeonia* have been discovered, but the number examined is still too small to permit any conclusions to be drawn as to their occurrence.

The attachment of chromatids end-to-end in the later stages of meiosis, as well as the fragmentation of chromosomes, is much more abundant in *P. Mlokozewitschi* than in *P. suffruticosa*. In 30 per cent of the anaphases and homoeotypic divisions fragments of chromosomes were found. These fragments were never seen during the metaphase, and numerous cells at the anaphase showed that they had just broken away, as in figure 3. They may consist of portions of a single chromatid or of the end of a chromosome, with two chromatid fragments side by side, though perhaps the former type, as shown in figures 3, 4, and 5, is more common. The break may occur at various places between the attachment constriction and the distal end of the chromatid. In figure 4 there are three fragments that have broken off at or near the attachment constriction and two small ones that contain only the distal end of a chromatid. In figure 3 the smaller fragment at the left has broken off about half-way between the attachment constriction and the distal end of the chromosome.

Fragmentation is considerably more abundant in cells which have an otherwise abnormal chromosome distribution. Of the anaphases and homoeotypic divisions in which five chromosomes were at each pole or on each spindle,

28 per cent showed fragments, while in those in which the division was into groups of abnormal number, there were 37 per cent of cells showing fragments. This tendency was the most marked in the group in which the heterotypic anaphase separation was into six and four chromosomes. Of the 19 cells in which this type of separation was observed, 9, or 47 per cent, showed the presence of fragments. In figure 3 there are six chromosomes at the left- and four at the right-hand pole of the spindle, while in figure 5 there are probably five chromosomes at the left- and three at the right-hand pole, though here the number cannot be made out with certainty. Two chromosomes, *A* and *B*, are completely outside of the spindle, in the cytoplasm.

During interkinesis the fragments may remain as masses of chromatin or

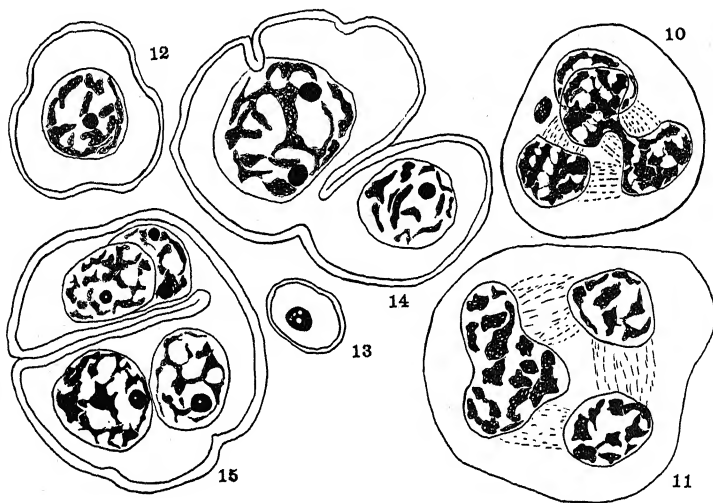


Fig. 10-15. Fig. 10. *P. Mlokozewitschi*, homoeotypic telophase, showing polycary and fusion of nuclei. Fig. 11. Later stage, a triad being formed. Fig. 12. Young, normal pollen grain. Fig. 13. Small grain formed by fragment. Fig. 14. Binucleate grain, formation of tetrad walls incomplete, one fusion nucleus. Fig. 15. 4-nucleate pollen grain, incomplete wall formation.

they may form small micronuclei. Occasionally, however, the remarkable phenomenon was observed of fragments with a small spindle fibre area between them, apparently at the anaphase of a separate mitosis, while the two main nuclei were in the interkinesis condition. This is illustrated in figure 6, while in figure 7 there are three small fragments with spindle fibre areas between them. That two sister chromatid fragments do not always go through this "mitosis" during interkinesis is made clear by the presence, in figure 8, of two quite similar fragments, obviously parts of sister chromatids at the homoeotypic metaphase. These have not separated at all and have not even formed a spindle fibre area around themselves. The fragments in figure 6 are quite long, as indicated by their depth of focus, and may include an

attachment constriction point, but in those on figure 7 there is less possibility of this.

The fragments persist throughout the homoeotypic division but rarely if ever are included in this process, being usually extruded into the cytoplasm. New fragments may, however, be formed at the homoeotypic anaphase, as in figure 9. At the homoeotypic telophase they may form extra nuclei (fig. 10), and the tetrads occasionally contain extra microcytes, the nuclei of which are too small to contain a whole chromosome. Figure 13 shows a small pollen grain formed by such a microcyte, the nucleus of which is in appearance like a single nucleolus, and is considerably smaller than any chromosome of this species.

The connection between the ends of sister chromatids, seen not uncommonly at the heterotypic anaphase, sometimes persists throughout the homoeotypic division. Figure 8 shows such a connection, stretching between the two spindles of the homoeotypic metaphase, while in figure 10 there is a bridge between two of the nuclei at the homoeotypic telophase, undoubtedly caused by the persistence of such a connection. During the later telophase, nuclei so connected become completely fused, so that a triad, instead of a tetrad, results. Figure 11 shows such a triad; the large irregular nucleus at the left is obviously the result of the fusion of two nuclei.

The young pollen grains of *P. Mlokosewitschi* show a number of abnormalities. Often giant pollen grains are seen with three or four nuclei. In these there is occasionally a large nucleus, obviously the result of fusion as described in the last paragraph, while there is sometimes a small micronucleus; but many times the grain is simply a four-nucleate tetrad, the walls of which have failed to be completed. Always there are furrows, as in figures 14 and 15, indicating the beginning of wall formation between the nuclei.

P. tenuifolia L. The material studied is of a horticultural strain growing in the Saunders garden. There seems to be little chance that the strain is the result of interspecific hybridization, or that such hybridization could be anywhere in the pedigree of this strain, since it has been cultivated. *P. tenuifolia* is a very distinctive species, unlike any other in the genus, and forms sterile hybrids with all other species that are cultivated at all. Hence one may logically assume that the cytological picture here presented represents the situation in the wild as well as in the cultivated strains.

P. tenuifolia is considerably more regular in its meiosis than *P. Mlokosewitschi*. Of 100 cells at the heterotypic metaphase, 29 per cent showed failure of pairing and the presence of two univalents and four bivalents instead of the usual five bivalent pairs. Out of 159 cells at the heterotypic anaphase and the homoeotypic divisions, only five, or 3 per cent, showed separation into groups of six and four chromosomes, while no other abnormal types of chromosome distribution were observed. In 13 cells, or 8 per cent, fragments were found. Again the percentage of fragments was higher in those cells in which the chromosome distribution was otherwise abnormal; of

the five cells in which the separation was into groups of six and four, two showed also the presence of fragments. Figure 16 shows a heterotypic metaphase with two univalents.

P. albiflora L. Three different cultivated strains of this species were studied: seedling no. 917, the variety "Lady Duff," and the variety "Silvia," which originated in the Saunders garden. No. 917 is a white single; the

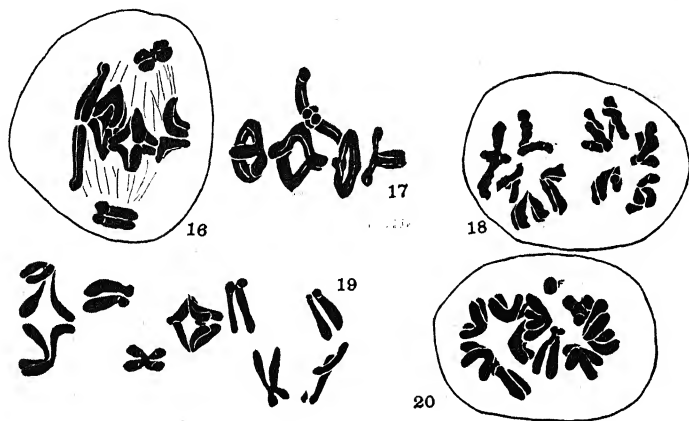


Fig. 16-20. Fig. 16. *P. tenuifolia*, heterotypic metaphase, showing asynapsis. Fig. 17. *P. albiflora*, var. "Silvia," heterotypic metaphase, non-disjunction (chromosomes slightly separated in drawing). Fig. 18. *P. albiflora*, "Lady Duff," homoeotypic metaphase. Fig. 19. *P. Smouthi*, early heterotypic anaphase, showing fragmentation at the right. Fig. 20. Homoeotypic metaphase of the same, showing a fragment, *F*, within the right-hand cell plate.

other two are semi-double varieties. "Silvia" is markedly distinct from the others in its tomentose carpels and its pronounced tendency toward polycarpy.

P. albiflora is more regular in its meioses than any other species studied. In no. 917, out of 68 anaphases and homoeotypic divisions studied, no abnormalities whatever were found. "Lady Duff" showed failure of pairing in 5 out of 100 pollen mother cells at the heterotypic metaphase. In 6 per cent out of 185 anaphases and homoeotypic divisions fragments were found, while in two of these the division was into groups of six and four chromosomes. In "Silvia" the irregularities were found more frequently. Of 100 heterotypic metaphases, 10 per cent showed two univalents, while of 74 anaphases and homoeotypic divisions, 11 per cent showed fragments and 3 per cent an abnormal chromosome distribution. In this variety, as well as in the other species, a chromosome pair was found near a pole of the spindle in occasional heterotypic metaphases, as illustrated in figure 17. This would lead to the distribution of six chromosomes to one pole and four to the other pole of the spindle at the heterotypic anaphase, and is, of course, true

cytological non-disjunction. Figure 18 shows a regular homoeotypic metaphase in "Lady Duff."

P. Smouthi Van Houtte (*P. albiflora* \times *tenuifolia*). This hybrid was first made almost 100 years ago (Van Houtte, 1845), and in the opinion of both Dr. Saunders and the junior author, the present horticultural strain is probably of the same clone as the original plant, since the hybrid is very sterile and is a difficult cross to make. Two different plants were studied, no. 6550 and no. 6582. No. 6550 was obtained from Old Farm Nurseries, Booskop, Holland, under the name of *P. anomala*, while the latter was bought from Barr and Sons, London, as *P. anomala* var. *intermedia*. Both, however, are identical in appearance with plants bought as *P. Smouthi* and fit the original description of this hybrid, as well as being decidedly intermediate in their taxonomic characters between *P. albiflora* and *P. tenuifolia*. They are certainly not *P. anomala*, since it is a widespread wild species and the plants here studied are completely sterile. Furthermore, their difference from the original description and the illustration of *P. anomala* (Gmelin, 1769, pl. 72) was clear after comparison of the two.

P. Smouthi is considerably more irregular in meiosis than either of its parents. Out of 65 cells at the heterotypic metaphase, 37, or 57 per cent, showed the presence of univalents, and in a number of cells these were four in number, with but three pairs of bivalents present. Nevertheless, only 5 out of 157 anaphases and homoeotypic divisions, or 3 per cent, showed an abnormal separation of the chromosomes. Fragments were found in 28, or 18 per cent, of these later stages. Some of the early anaphases showed the earlier stages of fragmentation particularly clearly. In figure 19, one chromatid of the lower chromosome of a pair has just broken at the attachment constriction. The fact that the broken chromatid crosses its sister, and has obviously paired at its end with the alternate chromatid of the homologous chromosome, indicates that a twist of the chromatids, or an asymmetrical chiasma (Sax, 1932), has occurred here. This is probably partly responsible for the fragmentation. Figure 20 shows a homoeotypic metaphase in which a fragment is contained within the right-hand cell plate.

The pollen of *P. Smouthi*, according to the tests of Dr. Saunders, contains 40-50 per cent of grains that appear good, but few if any germinate under the normal conditions of a germination test. The hybrid rarely sets seeds.

DISCUSSION

The most striking characteristic of meiosis in the forms studied is the general occurrence of the various abnormalities, not only in the known hybrid, but also in the species which are members of groups of closely related forms and in those which have no close relatives in the genus. A comparison of the frequency of the abnormalities in the various species should, however, shed some light on the underlying causes of them and on whether they are

of significance from the standpoint of the evolution of species within the genus. As a guide to this comparison, a table of the various frequencies, as described in the preceding section, is here given.

TABLE 1. *Percentage of frequency of meiotic abnormalities in four species and a hybrid of Paeonia*

	Percent- age of cells with univalents	Percentage of cells with abnor- mal separation	Percent- age of cells with fragments	Percentage of polyspory	Percentage of sterile pollen
<i>P. suffruticosa</i>	40	3(1)	4	6	10-30
<i>P. Mlokosewitschi</i>	48	17(8)	30	33	20
<i>P. tenuifolia</i>	29	3(0)	8	9	10-20
<i>P. albiflora</i>					
no. 917	X	3(3)	0	X	
"Lady Duff"	5	1(0)	6	X	5-15
"Silvia"	10	3(0)	11	5	
<i>P. Smouthi</i>	57	3(1.5)	18	21	50-60

Failure of chromosome pairing, or asynapsis. As underlying causes of asynapsis, the most significant are hybridization, "genetic-chromosome reaction" (Darlington, 1932), and the effects of the environment. The effect of hybridization in reducing chromosome pairing is now well known (Woodworth, 1929; Karpechenko, 1928; etc.). The action of genetic factors has been postulated by Darlington for a number of cases, many of them segregates from known hybrids, but the only one in which the factor is definitely known is *Zea Mays* (Beadle, 1930). Here a single recessive gene causes almost complete failure of pairing, although the number of bivalents formed is very variable in the asynaptic strain. Reduction of temperature may cause asynapsis, as in *Rhoeo discolor* (Sax, 1931).

Which of these underlying causes are the ones responsible for the condition in the various forms of *Paeonia* can be partly determined by a comparison of those here studied. The question of a genetic factor cannot be settled without studying more pedigreed forms, but in this connection it may be said that the rather general occurrence of asynapsis in the genus seems to indicate some more far-reaching cause than the action of a single gene, or even of a series of genes. As to hybridization, this is certainly the chief cause of asynapsis in *P. Smouthi*, as that hybrid shows an increase of 100 per cent over one, and a much greater increase over the other, of its parents. As to the species, their hybrid origin, at least in recent times, seems improbable.

The significance of asynapsis in the various species lies chiefly in the fact that it is correlated with their pollen sterility, while the other meiotic abnormalities are not. *P. Smouthi*, which has only 40-50 per cent of pollen grains that appear good, none of which will germinate, has also the highest percentage of asynapsis. *P. Mlokosewitschi* and *P. suffruticosa*, with about 80 per cent and 60-90 per cent of good pollen, respectively, are also next in

order in the percentage of asynapsis. *P. tenuifolia*, in which the pollen is 80–90 per cent perfect, has the next lowest percentage of asynapsis, while *P. albiflora*, with 80–95 per cent good pollen, the strongest of any, has the lowest percentage of asynapsis. This, of course, does not mean that pollen sterility is caused by the failure of chromosomes to pair at meiosis, but that the two phenomena are governed by the same agent.

Abnormal separation of the chromosomes, or non-disjunction. This phenomenon, as shown by the above table, is much less common than asynapsis and does not seem to be correlated with it. For instance, *P. Mlokosewitschi*, with a lower percentage of asynapsis than *P. Smouthi*, has more than five times as many gametes with an abnormal chromosome number, while *P. suffruticosa*, *P. tenuifolia*, *P. albiflora* var. “*Silvia*,” and *P. Smouthi*, with percentages of asynapsis ranging from 10 per cent to 54 per cent, all have the same frequency of non-disjunction.

Evidence from the heterotypic metaphases indicates that both asynapsis, as in figure 16, and the remaining of a bivalent pair at one pole of the spindle, as in figure 17, may be the immediate causes of non-disjunction. The former is “cytological” (Sax, 1932), the latter true genetic non-disjunction (Bridges, 1916). Both were recorded in *Uvularia* by Belling (1925) occurring together in the same anther, as they do in some species of *Paeonia*. Since the percentage of non-disjunction is in every form lower than that of sterile pollen, and in all but *P. Mlokosewitschi* much lower, and since no aneuploid forms of the genus are known, the genetic and evolutionary significance of non-disjunction is in *Paeonia* little or none. Extrusion of one or two chromosomes into the cytoplasm has been included as this type of abnormality in table 1, but separate figures for the frequency of this extrusion are given in parentheses.

Fragmentation. The very general occurrence of this phenomenon is perhaps the most unusual feature of meiosis in the genus. It is not correlated with asynapsis, as *P. Smouthi* has a considerably lower percentage of fragmentation than *P. Mlokosewitschi*, although the percentage of asynapsis is higher. There is, however, a positive correlation between fragmentation and the abnormal separation of the chromosomes. This is not brought out very clearly by the table, but is more evident from a study of different cells in the same species, as was mentioned above for *P. Mlokosewitschi*. One may conclude from this correlation that the disturbance which causes this abnormal separation is also partly responsible for fragmentation.

The best cases of fragmentation of the chromosomes during meiosis are those of *Tradescantia* (Darlington, 1929), *Chorthippus* (Bělař, 1929), and *Uvularia* (Belling, 1925). In the first, the fragments were believed to be formed during the prophase, since some cells at the heterotypic metaphase showed fragments and some did not. In *Chorthippus* and *Uvularia*, fragmentation occurs much as it does in *Paeonia*, by the breaking of the chromatids during the anaphase separation. The presence of a constriction prior to

this break could not be clearly determined, although many chromosomes at the late metaphase were markedly constricted near the fibre attachment point (fig. 19, at the left). This appearance is similar to the drawing out of the chromosomes observed during the anaphase by Bělař. The sticking together of chromosome ends is here, as in *Uvularia*, undoubtedly one of the immediate causes of fragmentation, but not the only one. In figure 19 a twisting of the chromatids is the immediate cause, although all such twists certainly do not result in fragmentation, as in many cases the chromatids of chromosomes that have just separated in the early anaphase are distinctly twisted and show no sign of it (cf. Sax, 1932, fig. 2a). For the breaking off of the end of a whole chromosome and the production of two sister chromatid fragments, as in figure 8, there must be still another condition, although this process could not be found while it was actually happening. These various types of fragmentation may all be simply accidental occurrences, but as pointed out above, the evidences of correlation seem to point to some disturbance which is the underlying cause of them all.

The other two abnormalities, the sticking together of chromosome ends, which can appropriately be called chromatid fusion, and the failure of wall formation in the tetrads, seem to be correlated with fragmentation and abnormal chromosome distribution, although no satisfactory data could be obtained on their distribution. For the latter abnormality, as found in *Kniphofia*, Moffett (1932) has postulated a partial failure of the spindle mechanism. This may be the case in *Paemonia* also, particularly since this partial failure would be just the type of disturbance that would cause the other abnormalities recorded. Whether a genetic-environmental reaction, as is apparently the case in *Kniphofia*, is here also the underlying cause can be accurately determined only after a careful study of more different species and hybrids, collected under many different conditions of environment.

Polycary and polyspory. These two phenomena, observed frequently in hybrids (Woodworth, 1929) and in species of large genera (Church, 1929), occur in *Paemonia* as a result of the extrusion of whole chromosomes, or parts of them, into the cytoplasm. The unusual feature of these phenomena here is that the extra nuclei and extra cells of the tetrads are formed more frequently from fragments than from whole chromosomes. This is shown by the size of the nuclei, as in figure 10, since they are generally smaller than a whole chromosome, and by the frequency of their occurrence. This, except for *P. Mlokoszewitschi* and *P. albiflora* "Silvia," corresponds, within the degree of probable error, to the percentage of fragmentation plus that of cells in which a whole chromosome has been extruded. The exceptions are very likely due to the fact that some of the fragments are so small that they become completely disintegrated during the homoeotypic telophase. From a comparison of the frequency of fragmentation and chromosome extrusion (table 1), one can conclude that 80 to 100 per cent of the micronuclei are formed from fragments.

The nucleus of the small pollen grains (fig. 13) is significant in its resemblance to the nucleoli of larger nuclei. This would tend to support the theory of Fikry (1930) that the nucleolus is a reservoir of chromatin material.

From a consideration of the above data, the conclusion can be reached that the two meiotic abnormalities which are of the greatest significance in regard to species evolution in *Paeonia* are fragmentation and chromatid fusion. In fragmentation, the fragments formed are of little significance, since they are usually extruded into the cytoplasm. Furthermore, no clones of *Paeonia* which regularly possess fragments, such as those of *Tradescantia* (Darlington, 1929), *Fritillaria* (Darlington, 1930), and other genera, have yet been discovered. More significant is the fact that the chromosomes from which the fragments have broken contain deficiencies. These would be transmitted to the offspring derived from gametes in the development of which fragmentation has occurred. That such gametes are viable is almost certain in *P. Mlokosewitschi*, since here the percentage of fragmentation exceeds that of sterile pollen. Both plants and animals with such deficiencies are known, as summarized by Darlington (1932), and in most cases morphological changes accompany them. A comparison of the lengths of the chromosomes of various species and races of *Paeonia*, to be undertaken in the near future, will be enlightening in this respect.

Chromatid fusion is significant in that it is the only mechanism yet observed in the genus for the formation of diploid gametes. A similar mechanism has been observed by Matsuda (1928) in *Petunia violacea*. Saunders (1933) has shown that all of the polyploid species will cross rather easily with at least one diploid species—i.e., *P. albiflora*. This shows that they are probably derived from the diploid species, and that therefore diploid gametes have functioned in their production. For these reasons one of the most fruitful lines of study in regard to the species formation in *Paeonia* will be a study of the reasons for and the frequency of occurrence and the causes of these abnormalities.

SUMMARY

1. Meiosis in four species and one hybrid of *Paeonia* is described. All are diploids with a chromosome number of $n=5$.

2. The following abnormalities of meiosis are found in all the forms studied: asynapsis, separation of the chromosomes into groups with abnormal numbers (non-disjunction in the broader sense), extrusion of chromosomes, chromosome fragmentation, end-to-end fusion of homologous chromatids, polycary, and polyspory.

3. Asynapsis is correlated in its frequency of distribution with pollen sterility. Both are most common in the hybrid, *P. Smouthi*. Abnormal separations, extrusion, fragmentation, and chromatid fusion are all correlated with each other in frequency of distribution, and are most common in *P.*

Mlokošewitschi, a member of a complex group of closely related species or subspecies.

4. Chromosome fragmentation apparently occurs during the early anaphase, and fragments may consist of parts of one or both sister chromatids. They are usually extruded into the cytoplasm and form extra nuclei.

5. Chromatid fusion results in the fusion of two nuclei at the homoeotypic telophase and the formation of diploid spores.

6. In regard to species formation in *Paeonia*, fragmentation is important in that it results in the production of gametes containing a deficiency in one or more chromosomes, and chromatid fusion is significant as a contributing cause of polyploidy.

In concluding, the junior author wishes to express his gratitude to Dr. A. P. Saunders for his cooperation and contribution of data throughout the work, to Mrs. G. C. Hicks and Dr. W. H. Spencer for their assistance in making available Dr. Hicks' slides and notes, and to Mrs. G. L. Stebbins, Jr., for assistance with the figures and the preparation of the manuscript.

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AERATION AND GROWTH OF CANTELOUP SEEDLINGS (*CUCUMIS MELO*)

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INTRODUCTION

For a number of years investigations have been in progress in this laboratory involving the growing of seedlings deprived of exogenous nourishment (cf. Pearl, 1927, 1928; Pearl, Winsor, and Miner, 1928; Pearl, Winsor, and White, 1928; Gould, Pearl, Edwards, and Miner, 1933). The technique used has been brought to a high degree of precision. It consists essentially in growing, in the absence of light, sterilized seeds on sterile agar gel in long glass test tubes, 2 cm. in diameter and 44 cm. long, closed by loosely fitting cork stoppers. This method makes it possible to standardize the environmental conditions to which seedlings are exposed to a point where individual variation is greatly reduced. The assurance of aseptic conditions and the ease of observation and measurement are also important items in its favor.

Early in the course of these experiments it was found that if the ventilation of the tubes was brought below a certain minimum by too tight stoppering abnormal seedlings resulted. Systematic experiments were undertaken to learn more about the effects and limits of the aeration factor, primarily with the object of perfecting the general seedling technique which was being used in the study of other general biological problems. Because of greater interest in other aspects of the work the results of these experiments have not hitherto been published, although their lesson was long ago incorporated into the routine seedling technique of the laboratory.

Although numerous experiments have been reported on the chemical influence of the atmosphere on germination and respiration, it appears that there have been few attempts to estimate their influence on growth in a systematic way. Wieler (1883) presents a good account of the work on this subject prior to 1883.

Wieler (1883) placed plants in a bell jar which could be evacuated to obtain low oxygen tensions and which could be refilled with hydrogen and evacuated again in order to secure still lower tensions. The elongation of seven kinds of seed plants and the growth of three species of fungi were measured with a horizontal microscope, and the oxygen tensions that just suppressed or permitted growth were ascertained for each species. He found that as he exposed plants to successively lower oxygen tensions the growth rate increased to a greater rate than in air; but with still greater rarefaction

of the air the rate decreased to zero. More recently Heumann (1923), using essentially the same procedure, was able to confirm this observation. He considered that the three monocotyledonous plants which he tested elongated more rapidly by virtue of an increased rate of cell division in the growing zones of the leaves; the two dicotyledonous plants, while showing maximal growth in reduced air pressure, did not exhibit a higher division rate.

Because of the fact that there have been few attempts to measure the course of seedling growth under diverse conditions of aeration, it has seemed desirable to publish some of our old results at this time, and also to extend their scope with some further experiments, which have been done during the current academic year.

EXPERIMENTS AND RESULTS

The first series of experiments to be reported here consisted of daily measurements of the hypocotyl length of 50 canteloup seedlings in individual tubes stoppered in five different ways which might be supposed to retard gas diffusion to different degrees. In the second series, in comparison with tubes closed by loosely fitting cork stoppers, growth was measured in tubes in which the air was almost completely renewed daily. To anticipate the results briefly, it may be said that although by tightly closing the tubes it is possible to retard growth greatly and to induce conspicuous abnormalities in growth, nevertheless the loosely fitting cork stoppers, or loosely packed cotton plugs, one or the other of which methods is regularly used, permit enough gaseous diffusion to meet the needs of canteloup seedlings. Additional ventilation does not have any measurable influence on seedling growth or the utilization of food materials.

Series A

Fifty of the heaviest seeds from a single canteloup (*Cucumis melo*) melon were freed of their seed coats, sterilized by soaking one minute in 1/1000 HgCl₂ solution, and soaked for three hours in distilled water before being transferred to individual culture tubes containing 40 cc. each of 1.3 per cent agar which had been previously sterilized in a steam autoclave. The tubes were kept in incubators at 30°C. in darkness. Five types of stoppers were provided. Tubes 1-10 (treatment I) were closed with loosely wadded cotton plugs, following the practice of bacteriologists; the next ten tubes of treatment II received loosely fitting cork stoppers; nos. 21-30 (III) were provided with tightly fitting cork stoppers, and to distinguish further this method from the foregoing one, after the stoppers were in place they were sealed with melted paraffin. Tubes 31-40 (IV) received rubber stoppers sealed in place with paraffin. It was difficult to get the rubber stoppers firmly sealed, and it was not until the third day after planting that the tubes were really effectively sealed. Tubes 41-50 (V) received two-hole rubber stoppers fitted with two short glass tubes lightly plugged with bits of cotton. The seedling in tube 45 was abnormal in several respects and was not used in considering the results.

The immediate result of these five treatments was the appearance of two types of seedlings. The first, second, and fifth treatments, or those in which gas interchange was permitted, resulted in tall, slightly tapering seedlings of the type that grow rapidly and regularly, approaching an upper growth limit and remaining without further visible change for an extended period until death occurs. This type of seedling has proved to be highly desirable for experimentation. The second type of seedling appeared in tubes in which

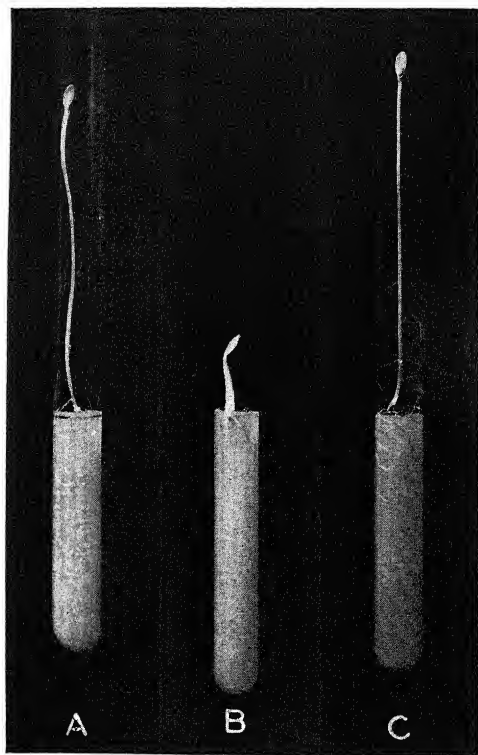


Fig. 1. Seedlings of *Cucumis melo* grown as described in text. A. Normal seedling, cotton stopper, 18 days after planting. B. Dwarfed seedling, sealed cork stoppers, 19 days after planting. C. Normal seedlings, loose cork stopper, 18 days after planting. $\frac{1}{4}$ natural size.

an effort had been made to exclude the possibility of gaseous diffusion. These seedlings were short, with conspicuously thickened hypocotyls and larger cotyledons than the others. The roots were thickened and twisted into spirals, and lay on the surface of the agar with very little penetration.

The gross appearance of the two types of seedlings is shown in figure 1.

Details regarding the growth of the seedlings in these experiments are given in figure 2 and table 1.

Differences in height within this series of experiments were marked. In

figure 2 mean heights are represented as ordinates and time (in days) along the abscissal axis, each treatment being represented by a separate curve of the logistic type. Two of these curves run almost the same course, growth in tubes closed by loosely fitting cork stoppers being only slightly slower than in the cotton-stoppered tubes. The difference between I and II is so small that no importance can be attached to it. The final height, 14.2 cm., is about the value usually found in tests of canteloup seedlings grown with these methods at 30°C. Lagging somewhat behind, both in growth rate and in final height, are the seedlings where gas exchange took place through two

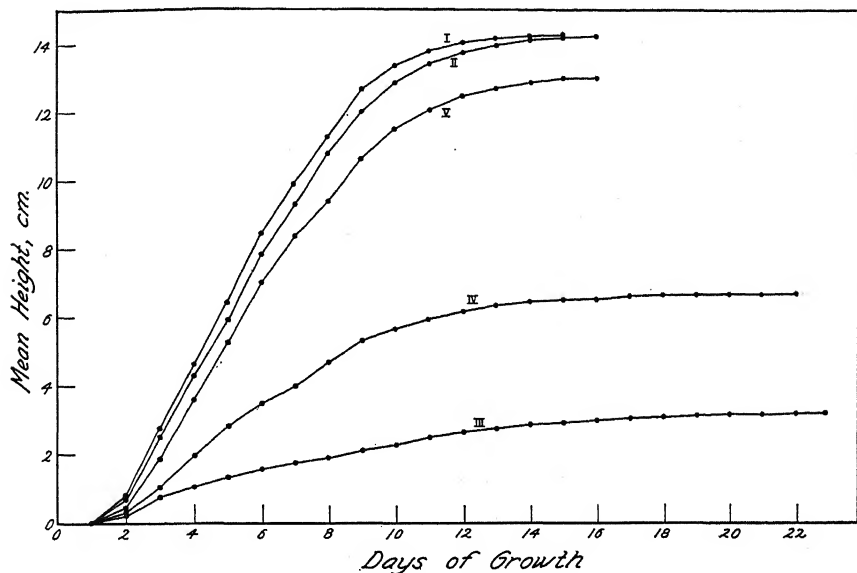


Fig. 2. Mean hypocotyl heights of *Cucumis melo* seedlings grown in tubes closed in different ways as follows: I, cotton plugs; II, loosely fitting cork stoppers; III, sealed with paraffined corks; IV, partly sealed with rubber stoppers; and V, two cotton-plugged glass tubes in rubber stoppers.

cotton-plugged glass tubes set in a rubber stopper. Measurable growth persisted for a longer time than in the preceding two treatments. As has been noted, leakage occurred around the rubber stoppers of treatment IV intended to prevent diffusion entirely, so that during the first three days these seedlings received more air than was contained in their tubes. This partial shortage in air supply resulted not only in slowing growth and in prolonging it far longer than in the more vigorous cultures, but it yielded seedlings about half as tall (6.7 cm.) as those in the best cultures. The volume of these cultures is about 135 cc., and after making allowance for 40 cc. of agar and the space occupied by cork stopper, an effective air space of 92 cc. is left. The most successful efforts to limit the air supply to this volume, which were

in those tubes in which the cork stoppers were sealed in with paraffin, limited growth to an even greater degree than before. This group attained a mean height of only 3.2 cm.

Careful study of these curves shows that while they are all asymmetrical, a disproportionate amount of the total growth occurring in the first half of the growth cycle, the curve for the seedlings grown in tightly sealed tubes has the greatest degree of skewness. They attained about half of their final height in the first quarter of the period of measurable growth.

TABLE I. *Observed values for nine measured variables in the experiments of series A*

Treat- ment	A Final height (cm.)	B Duration of growth (days)	A/B Growth rate (cm. day)	C Dura- tion of life (days)	D Mean fresh weight (mg.)	Method of closing tubes
I	14.27	14.0	1.02	18.5	510.4	Cotton plugs
II	14.23	14.8	0.96	21.2	484.5	Loosely fitting corks
V	12.99	14.9	0.87	18.2	523.8	Glass tubes in rubber stopper
IV	6.70	17.1	0.39	31.7	423.4	Rubber stopper sealed after 3 days
III	3.21	18.4	0.17	39.4	368.6	Sealed, cork and paraffin

	E	F	G	H	I
	Mean dry weight in mg.				
Treatment	Cotyledons	Hypocotyls	Roots	Roots + hypocotyls	Whole plant
I	6.04	9.14	4.11	13.25	19.29
II	6.05	8.75	3.00	11.75	17.80
V	6.19	8.93	3.37	12.30	18.49
IV	6.26	7.47	2.17	9.64	15.90
III	6.95	6.32	1.29	7.61	14.56

When we turn to the time variables (duration of growth period and total duration of life), a different picture presents itself, but one which stands in a simple, consistent relation to that seen in the size and growth variables. It will be seen from columns B and C that the duration of the growth period and the total duration of life become longer as the cultures are more and more imperfectly aerated, being shortest under treatment I and longest under treatment III. It is of interest to examine another function, the growth rate, in order to emphasize this point. To have a rough measure of rate, satisfactory for comparative purposes, the final heights (column A) were divided by the durations of measurable growth (column B). These quotients decrease with decreases in aeration. Thus, it appears that under the conditions of these experiments low growth rates are definitely connected with long life, supporting the conclusion reached in another place (Pearl, 1928) that the length of life depends inversely on the rate of living.

If the time rate of growth (column A/B) and the total duration of life (column C) for each individual seedling are correlated, the resulting co-

$$r = -.854 \pm .025.$$

But this is not quite a dependable result, for two reasons: In the first place, the length of the growing period not only itself forms a considerable fraction of the total duration of life, but is also involved in the computation of

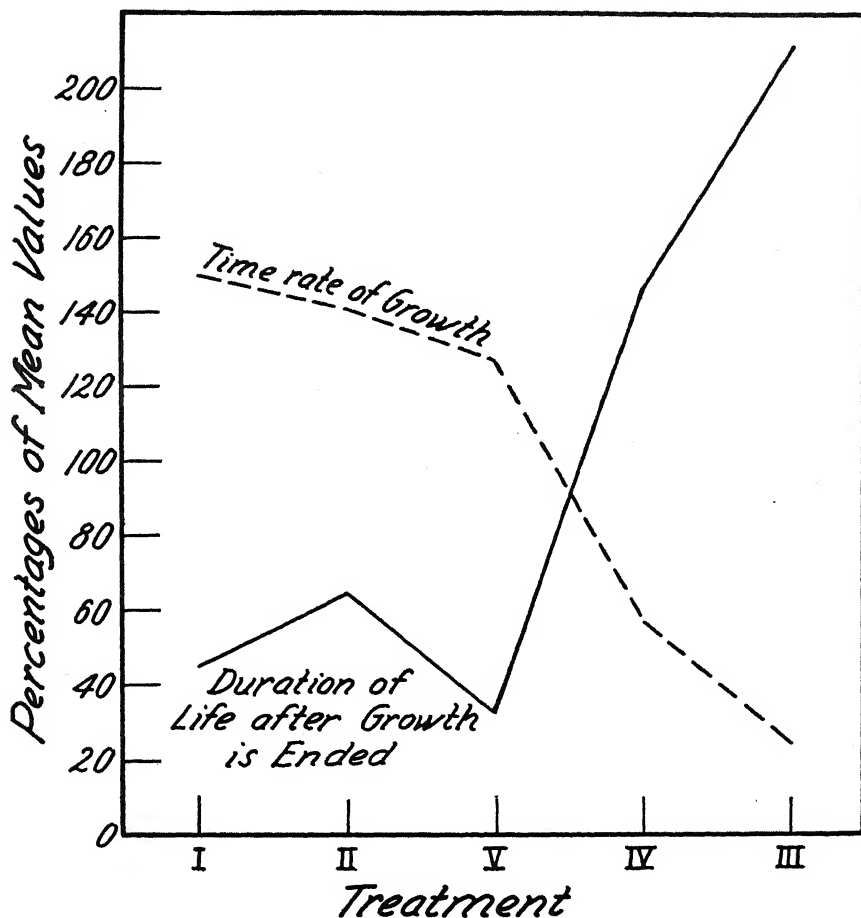


Fig. 3. Relationship between time rate of growth and duration of life after growth is ended. For further explanations see text.

the rate of growth. Consequently there is the probability of an element of spurious correlation being present in the observed coefficient. In the second place, there is a definite discontinuity in the material between the better and the worse ventilated series.

A much more significant relationship is that between rate of growth, on the one hand, and duration of life *after* the end of the growth period (column C—column B), on the other hand. On the “rate of living” theory of life

duration these two variables should show an inverse relationship to each other. The more rapid the time rate of growth, the shorter the duration of life after growth is ended, is the expectation in a closed system without exogenous nourishment such as characterizes the organisms in these experiments; and vice versa. Just such a relationship was observed experimentally and is shown graphically in figure 3. In this diagram both variables are put upon a relative basis by equating the means of each to 100 per cent and expressing all observed values as percentages of their respective means.

One further set of observations in table 1 merits discussion, namely the mean dry weight of the cotyledons at the beginning of death—i.e., when the plants showed the first symptoms of beginning disintegration. This measurement is an important one because it indicates the degree to which stored food materials have been removed from the cotyledons. This process went on most efficiently in the best-aerated seedlings, as shown by the lightest dry weight; and least efficiently in the thoroughly stoppered tubes. If the dry weights of the entire plants, given in column I, are reexamined, it will be seen that the lowest mean was that for treatment III. The inference to be drawn seems to be that the character of the respiratory process under treatment III was such as to be unusually wasteful of food materials. What is known of respiratory processes where the anaerobic phase dominates is in accord with this inference.

Series B

In this series of experiments there were two groups of seedlings: viz., (1) normal controls, of which there were 10, and (2) forcibly aerated, of which there were 8. In the control group the tubes were closed with the usual loosely fitting cork stoppers (as in treatment II in series A). In the forcibly aerated group each tube was closed by a rubber stopper through which passed two glass tubes, one reaching nearly to the surface of the agar and the other just passing through the stopper. At the time of the daily observations on hypocotyl length, from 80 to 90 cc. of air washed through 1:1000 mercuric chloride solution was forced into each tube of the second group. Since the air space in a culture tube amounts to about 90 cc., this provided a fairly complete renewal of the atmosphere of the tube. The other conditions of the experiment were the same as in series A. The 1 per cent agar used in the culture tubes was made up in Knop's solution instead of in distilled water. Measurements were made in red (non-actinic) light. When growth ceased the cotyledons were cut off, and the fresh and dry weights of these and of the remainder of the plant (hypocotyl + roots) were recorded. The mean weight of the seeds planted in the aerated group was 23.1 mgm.; of those planted in the stoppered-tube control tests, 23.7 mgm.

The mean heights of the two groups of seedlings are presented in graphical form in figure 4. The seedlings in the stoppered tubes grew slightly slower than the forcibly aerated group, but reached a slightly greater final height. Neither difference is significant numerically, considering the degree of varia-

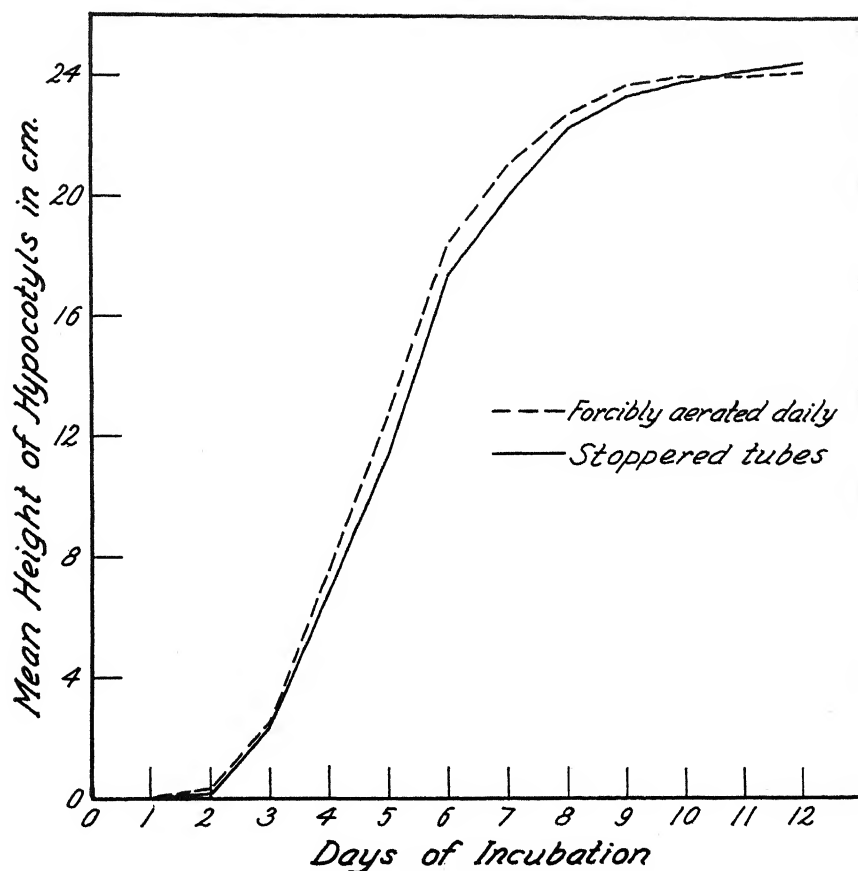


Fig. 4. Growth of hypocotyls in experiments of series B.

tion of the individual values. The greater final heights and weights of the seedlings of series B, as compared with those in series A, must be attributed to the presence of mineral salts in the agar.

The fresh and dry weights of the cotyledons, and of the roots and hypocotyl combined, together with the corresponding percentages of dry matter, are presented in table 2.

TABLE 2. Mean weights of seedlings in series B experiments at end of growth

	Mean fresh weight (mgm.)	Mean dry weight (mgm.)	Percentage of non-aqueous matter
<i>Hypocotyl and roots</i>			
Forcibly aerated	938.8	17.9	1.91
Stoppered	939.7	17.4	1.85
<i>Cotyledons</i>			
Forcibly aerated	92.9	6.5	6.98
Stoppered	118.6	7.1	6.02

Plainly there is again no significant difference in performance between the seedlings of the two groups. It may therefore be concluded that under the conditions of these experiments closing the culture tubes with loosely fitting cork stoppers did not have any injurious effect on the growth of the seedlings.

SUMMARY

The results of the experiments described in this paper may be summarized as follows:

1. Seedlings of *Cucumis melo* grown under aseptic conditions in the dark without exogenous nourishment show a diminished growth rate, lower final height of the hypocotyl, and less efficient metabolic translocation of food materials from the cotyledons to the growing plant as the ventilation of the tubes in which they are grown is progressively less adequate.

2. At the same time the mean duration of life of the seedlings increases as the ventilation is made progressively poorer.

3. The above results are interpreted as accordant with, and therefore in so far confirmatory of, the rate-of-living theory of life duration.

As a matter of practical experimental technique the closing of the seedling tube with either a loosely fitting cork or cotton plug permits entirely adequate ventilation for normal growth and life duration.

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A PRELIMINARY STUDY OF VARIETAL RESISTANCE IN
THE PINEAPPLE TO THE ROOT ROT FUNGUS
*NEMATOSPORANGIUM RHIZOPHTHORON*¹

ERNST VON KESSELER (Deceased Aug. 29, 1933)

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Root diseases of pineapple plants in the Hawaiian Islands caused by several fungi are among the factors making for irregularity of growth and poor productivity. Their actual importance has not been satisfactorily determined because, until recently, injuries caused by mealy bugs have been confused with results of root destruction. Still it is clear that root rot is of major importance in at least some fields, and there is no immediate prospect for direct control. Consequently, in view of an extensive pineapple breeding program being carried on by this Station, it seemed essential to learn something of the relative resistance of pineapple varieties and hybrids to root rot and to develop suitable technique for varietal comparisons.

According to Sideris and Paxton (1931), there are three distinct types of root rot of pineapple, of which the rapid soft rot caused by pythiaceous fungi is most important. These writers recognized twenty-three species of *Pythium* and *Nematosporangium* as causes of soft root rot. For obvious reasons in this present study it was essential to concentrate attention upon one pathogen. *Nematosporangium rhizophthoron* Sideris (1931) was the fungus chosen, and a subculture of Sideris' type culture was used throughout. This fungus was regarded by Sideris and Paxton (1931) as perhaps the most important of the group from the standpoints of both aggressive parasitism and wide distribution in pineapple fields.

Three very different measures of susceptibility have been attempted in this work, as follows: rate of progress of rotting in very young root tips in soil during 48 hours after inoculation; percentage of roots rotted in inoculated water culture during a longer period of 21 to 31 days; and retardation of plant growth in infested soil during a still longer period. The last named is a measure of all resistance factors combined and would seem to be a direct measure of practical resistance. Therefore, it appeared to be an important first method to use in this study of varietal resistance, but the slow growth of pineapple plants and their extremely slow reaction to injuries presented difficulties which prevented its extensive use. Measurement of the rate of invasion of young root tips, on the contrary, takes into account only part of

¹ Technical Paper No. 56 of the Experiment Station of the Association of Hawaiian Pineapple Canners, University of Hawaii.

the possible factors in resistance, but it proved to be a method sufficiently rapid and precise to give results of statistical significance, and therefore was used more extensively. As for counts of roots rotted in infested water culture, it is thought that this gives a measure chiefly of susceptibility of roots to entry by the pathogenic fungus.

SINGLE ROOT INOCULATION

Root observation boxes of the size $3\frac{1}{2}$ by 13 by 17 inches with movable glass walls on both sides, described by Dean (1923) as "small boxes," were used as containers for the plants to be tested. These boxes, filled with soil, were sterilized by autoclaving 4 hours twice during 72 hours, with an interim of one day between the sterilizations. The pineapple propagating materials, whether crowns, shoots, or slips, were then planted, two, three, four, or sometimes five in one box, according to the size of the plant material. The crowns of the variety "Cayenne" were always planted three in one box. The propagating material represented exclusively crowns in the case of Cayenne and lot 520; but slips and seldom shoots in the case of the other varieties.

Three to five weeks after the planting, sometimes even longer, the roots had grown through one-half to three-fourths of the depth of the box, which represented the appropriate stage for inoculation. Only roots of normal appearance growing for 6 to 7 cm. along the glass walls were used.

The inoculation method used was the following: Mycelium grown 24 hours on agar² in a Petri dish at 25° to 28° C. was cut into pieces about one centimeter square. The glass sides of the root study boxes were removed, and the agar squares with fungus were carefully placed against the tips of the roots in such a way that the growing margin of the colony lay 2 to 3 millimeters in advance of the root tip, thus leaving 7 to 8 millimeters of the root tip covered with fungus. Then the glass walls were carefully replaced. After 48 hours the walls were removed again, the inoculated roots were cut off about 10 cm. above the tip, carefully washed, and then examined. The reason a 48-hour interval between inoculation and observation was chosen was because on the one hand this proved to be long enough to obtain a measurable rot; and on the other hand it did not allow the fungus to proceed into hardened tissues of the root, which might retard the rate of further rotting.

The examination of the roots to determine the extent of rotting offered considerable difficulty. In some cases the diseased tips were browned, and the length browned agreed with the length invaded by the fungus as indicated by microscopical observation. In the greater part of cases, however, the discoloration was either slight or entirely absent in freshly invaded and softened tips. Consequently, direct observation was not adequate. Since the great

² The medium recommended by F. P. Mehrlich was as follows: KH_2PO_4 , 1 g.; MgSO_4 , 0.5 g.; peptone, 1 g.; malt extract, 5 g.; dextrose, 15 g.; agar, 20 g.; water, 1 liter.

number of roots to be investigated did not allow the long process of microscopical examination, a shorter method of determining the extent of disintegrated tissue was necessary. Stains, including methyl blue, methyl violet, Gram's iodine, and erythrosin, were tried to facilitate macroscopic observation, but without satisfactory results.

Another method which proved much better was based upon the softening and disintegration which follow invasion by this fungus. The root to be tested was grasped at its basal end with the fingers of the left hand. With broad forceps held in the right hand the root was then gripped gently with slight pressure near its base. With a downward movement of the right hand, the forceps first slipped along the root towards its tip until softened tissues were encountered. Here the forceps broke the affected tip from the sound base of the root. The length broken off was then measured.

In spite of a variable personal element, this method seemed a good one. The point of breaking coincided rather closely with the margin of disintegration as observed microscopically. Furthermore, in browned roots the length browned agreed closely with the length broken off, as shown by the tests recorded in table I.

TABLE I. *Comparison of methods of measuring extent of rotting of pineapple root tips. Lengths browned and lengths broken off, in millimeters, are reported for selected roots in which browning was apparent*

Smooth Cayenne		Ruby		Hybrid lot 520		
Browned	Broken off	Browned	Broken off	Browned	Broken off	
17	19.5	10	11	11	12	
20	19	6.5	5	10.5	10	
15	15.5	9	8	12	11	
17	15	11	12	10	10	
18	16	8	8	9.5	10	
23	21			8.5	9	
15	15			19.5	17.5	
20	18.5			13	14	
14.5	15.5					
16	16					
20	20					
19	18.5					
16	17					
19	20					
Totals	249.5	246.5	44.5	44.0	94.0	93.5

It must be stated that healthy root tips break in some cases, but then only the very tip breaks off. No cases were on record on a check test where the length of the broken off tip exceeds 7 mm., whereas the length of the broken off part of inoculated roots in very few cases is lower than 7 mm. In the variety Cayenne, for instance, where the greatest number of roots were investigated, only 15 roots out of 770, or 1.95 per cent, ranged below 7.5 mm. When 22 healthy white root tips of Cayenne were tested the lengths broken off were as follows (mm.): 2, 5, 0, 3.5, 0, 4, 0, 6, 0, 7, 0, 5, 0, 0, 3, 2, 7, 0,

0, 3, 1, 0. These tests of the method and, still more, the actual varietal comparisons presented below, make it plain that the method is sufficiently accurate to yield statistically significant results when adequate numbers of roots are tested. It is recognized by the writer that this is more strictly a measure of disintegration of tissue than of invasion by the fungus. In one variety there is probably a rather uniform relationship between invasion and softening, but this may not be uniform between varieties. Table 2 represents the

TABLE 2. *Statistical analysis of the average length of root of pineapple varieties and hybrids rotted in 48 hours*

Variety	No. of plants investigated	No. of roots investigated	Mean rot (mm.)	Standard deviation	Coefficient of variability
Cayenne	106	770	18.08 ± 0.106	4.36 ± 0.075	24.12 ± 0.415
Clon 8705 (Cross from Cayenne X Smooth Guatemala)	11	89	17.04 ± 0.199	2.78 ± 0.141	16.32 ± 0.825
Clon 8841 (Cross from Cayenne X Ruby)	11	50	16.92 ± 0.301	3.16 ± 0.213	18.67 ± 1.260
Wild Kailua	148	619	16.73 ± 0.098	3.76 ± 0.069	22.47 ± 0.430
Clon 7789 (Cross from Cayenne X Queen)	59	437	16.46 ± 0.119	3.69 ± 0.084	22.42 ± 0.511
Clon 8597 (Cross from Cayenne X Pernambuco)	18	94	16.08 ± 0.148	2.04 ± 0.100	12.66 ± 0.623
Pernambuco	106	489	15.74 ± 0.110	3.62 ± 0.078	23.00 ± 0.496
Congo *	13	40	15.00 ± 0.447	4.19 ± 0.319	27.93 ± 0.216
Ruby	87	331	14.83 ± 0.156	4.21 ± 0.110	22.34 ± 0.580
Natal	134	558	14.57 ± 0.097	3.39 ± 0.068	23.26 ± 0.470
Taboga *	17	64	14.53 ± 0.386	4.58 ± 0.273	31.56 ± 1.881
Lot 520 (F ₁ hybrid, Cayenne X Wild Brazil)	100	500	12.69 ± 0.124	4.10 ± 0.087	32.31 ± 0.689
Wild Brazil *	8	27	10.63 ± 0.398	3.08 ± 0.283	28.93 ± 2.656

* The values for the varieties Congo, Taboga, and Wild Brazil are not based on a sufficient number of measurements; these values have, therefore, to be considered with caution.

results of the single root inoculations performed from October, 1931, to July, 1932.

The results given in table 2 indicate the relative speed with which rotting occurs. The varieties are listed according to their average amount of rotting in 48 hours, and therefore according to the speed at which the rot proceeds. Cayenne stands at the first place as most susceptible, and Wild Brazil stands last.

Some varieties, as Congo, Taboga, and Wild Brazil, lack sufficient numbers of investigations and the values given cannot be considered as ultimately reliable. However, they will give some indication about the behavior of their roots.

The high coefficients of variability in these tests are of course due to deviations in the lengths rotted. These deviations, in general, were distributed equally over the measurements of each single plant. Only in rare cases the mean values of single plants within one root box varied considerably from one to another, thus perhaps indicating different individual plant responses to the fungal attack.

It is interesting to note that in the hybrid varieties, in which both the parent types had been tested as in the cases of lot 520, clon 8841 and clon 8597, the values of the mean rots of these hybrid varieties lie in between the mean values of their parent varieties. To draw further conclusions, however, would be premature.

INOCULATION ON ROOTS IN WATER CULTURE

Pineapple plants grown in jars of tap water were inoculated in several ways in an attempt to obtain simultaneous infection of many or all the root tips to provide another means of measuring rate of rotting of roots. This was not wholly successful, but during these attempts it was learned that the percentage of roots infected was not uniform between varieties. Consequently, two series of varietal comparisons were made. The plants were grown in liter jars of tap water for about 3 to 4 weeks until roots had reached a length suitable for inoculation. Nutrient solutions were tested in comparison with tap water, but in addition to their being less convenient they reduced somewhat the percentage of infection.

Inoculation was accomplished by adding to the water in each jar one-third of a 24-hour-old fungus culture grown on agar at 25° to 28° C., cut into pieces about one centimeter square. Adding the mycelium grown on hemp seeds in water was less convenient and presented no advantage. The use of zoospores³ was also tested but found unsatisfactory.

Data taken 4 weeks after inoculation consisted, in the first series, of counts of healthy and rotted roots. In the second series other data were recorded as well. Tables 3 and 4 present the results of these two tests.

In the first test (table 3) if the percentage of rotted roots is considered as an indication of the susceptibility of each variety, Pernambuco will rank as the most susceptible, followed by Wild Kailua, Cayenne, lot 520, and finally Natal as the most resistant.

The result of the second test (table 4) is entirely different. If the percentage of rotted roots is considered as a criterion of susceptibility, Cayenne

³ Zoospores were obtained only with difficulty at first, but the following method produced them abundantly: A piece of fungus culture on the agar medium was transferred to double distilled sterile water, and a hemp seed which had been boiled until the seed coat burst was added. This culture was then incubated at 27°C. for 8 days. After that time the fungus had grown extensively and developed an abundance of zoosporangia. Then the fungal mat was transferred to double distilled water and then placed in the ice box for 30 to 40 minutes. After 2 to 3 hours zoospores in abundance appeared.

TABLE 3. *First inoculation test of 5 different varieties in water culture extending from January to June, 1932*

Variety	Propagating material	No. of plants	No. of roots	Mean number of roots rotted (%)
Cayenne	Crowns	27	819	58.7
Lot 520	"	21	243	54.4
Wild Kailua	Slips	17	344	64.9
Pernambuco	"	18	299	92.9
Natal	Shoots	11	171	53.7

ranks first by far, followed in descending order by Natal, Wild Kailua, Congo, Ruby, Queen, Bermuda and, finally, Pernambuco as the least susceptible. The plant weight increase, in per cent, which unpublished data of the author show to be closely correlated in the Cayenne variety to both the percentage of healthy roots and the total number of roots formed, does not show this relationship in the present case where different varieties are compared.

TABLE 4. *Second inoculation test of 8 different varieties in water culture, tested simultaneously from June to August, 1932*

Variety	Propagating material	No. of plants	No. of roots	Mean no. of roots rotted (%)	Initial weight (gms.)	Final weight (gms.)	Increase weight (gms.)	Increase weight (%)
Cayenne	Crowns	24	424	65.4	72.7	92.0	19.4	26.4
Wild Kailua	Slips	24	173	40.7	76.0	81.2	5.8	8.1
Congo	Slips	21	80	29.4	62.6	60.1	-2.5	-2.3
Natal	Shoots	22	202	55.0	101.0	101.0	0	— .2
Ruby	Shoots	23	156	23.6	66.8	84.3	17.4	26.7
Bermuda	Slips	8	80	12.5	59.0	72.7	13.7	24.4
Queen	Shoots	21	266	21.1	83.1	105.2	22.1	27.1
Pernambuco	Slips	19	118	10.1	79.8	94.0	14.2	18.2

These unpublished data on Cayenne have shown that when plants (crowns) from different fields in different localities are compared, there are great differences in rates of rotting which are thought to be related to differences in composition of the plants. In the present case the great deviations between the first and second water culture tests are believed to be due to the fact that the first test was made with plant material held too long after picking and perhaps dried too much before being placed to root. Many of these plants produced no roots at all. The last test, however, was made with fresh plant materials, collected right after the parent plants had fruited, and planted as soon as they had cured adequately. These rooted freely. So it may be fair to consider the second set of investigations as the more reliable.

RETARDATION OF GROWTH IN INFESTED SOIL

Preliminary tests in experimentally infested soil failed to reveal any great retardation of plant growth as compared with uninfested soil. The follow-

ing more extensive experiment was therefore made, comparing two types of soil to determine the possible limiting effect of the soil customarily used in local glass house work. Soil no. 1 was from a region of high rainfall (about 80 inches per year) and was compact, highly colloidal, and of low pH.⁴ Soil no. 2 was the soil customarily used, from a region of lower rainfall (about 50 inches). It was of better structure and higher pH.⁴ Soil no. 1 was from a field where another phycomycete, *Phytophthora cinnamomi* Rands, develops destructively; soil no. 2 was from a district relatively free from such fungi. Both soils were mixed with ground peat-moss in the proportion of 5 soil to 1 peat-moss (volume) to improve drainage and allow better root penetration. The inoculum used was the fungus grown 6 to 8 days in Johann's (1928) corn meal and sand medium.

The plant containers used in this study were wooden boxes measuring 5½ by 16 by 19½ inches, with one removable side wall. They were filled with soil, then sterilized by steaming 2½ hours, and were then ready for infestation. This was accomplished by removing the side wall and replacing about 1½ inches of soil with the inoculum at about two-thirds of the depth of the box. Two plants were then planted in each box. Equal numbers of boxes were infested and left uninfested as checks. The test was started on January 9 to 11, 1932, and the measurements were taken from July 5 to 12, 1932. The results are given in table 5.

No essential differences are apparent, with the exception of Cayenne in soil no. 1, where the check plants showed a weight increase of 308.4 per cent of the initial weight but the inoculated ones, only 256.8 per cent. The significance of this difference is indicated by the fact that only one plant in infested soil showed as high a percentage gain as the mean of the check plants, and that only two check plants failed to gain more than the mean in the infested soil. Under the same conditions Pernambuco and lot 520 showed no appreciable differences. This probably indicates disease resistance in those varieties and susceptibility in Cayenne. In soil no. 2 no appreciable differences could be noted even in Cayenne, indicating apparently that the soil type limits the parasitism of this fungus.

DISCUSSION

Considering the tests made certain definite differences seem to exist in the susceptibility of different pineapple varieties to *Nematosporangium rhizophthoron*. Cayenne stands highest in susceptibility in the single root inoculation test and in the second water culture test. Wild Kailua ranges second and third in these two tests. Pernambuco, Congo, and Ruby range fairly low in susceptibility in both the tests mentioned. These two tests disagree only in regard to the variety Natal, which ranges low in susceptibility in the single root test and high in the second water culture test.

⁴ These determinations were made by F. A. E. Abel with the hydrogen electrode at the conclusion of the experiment: no. 1, pH 4.43; no. 2, pH 5.69.

TABLE 5. *Plant growth in infested and uninfested soil in 6 months. Weights given are mean values in grams.*

Plants	Crown	Soil	Initial weight	Final weight		Root weight as percentage of initial weight	Increase in total weight	
				Total	Roots		Amount	Percentage of initial weight
					Top			
Cayenne	10 crowns	No. 1, infested	106.9	382.1	32.9	349.2	266.1	256.8
	" "	check	101.7	397.5	45.2	352.3	306.9	308.4
	" "	No. 2, infested	97.8	271.9	16.5	255.4	173.1	194.5
Pernambuco	" "	check	103.7	288.4	21.4	267.0	185.7	183.9
	4 shoots	No. 1, infested	198.0	533.7	65.7	468.0	336.7	169.5
	" "	check	143.7	380.5	19.5	361.0	236.7	170.7
	" "	No. 2, infested	178.0	399.0	19.2	379.7	221.0	150.5
Lot 520	" "	check	183.7	402.2	26.7	392.2	246.0	156.7
	6 crowns	No. 1, infested	208.1	533.2	29.2	469.0	346.5	172.4
	" "	check	211.9	595.2	64.8	500.3	353.2	172.9
	" "	No. 2, infested	183.7	402.2	26.7	392.2	216.7	123.7
	" "	check	221.6	595.7	36.8	468.8	284.2	129.2

Hybrid lot 520 ranges very low in susceptibility according to the single root test; but Wild Brazil is still lower, according to the small number of tests made on this latter variety.

The three hybrid varieties, of which the parent forms were tested, as lot 520, clon 8841, and clon 8597, ranged intermediate between the respective parent forms according to the single root inoculation test.

The soil type is apparently of considerable importance for activities of the fungus *Nematosporangium rhizophthoron*. Since the soil which came from a high rainfall region showed considerable differences in growth between inoculated and check plants of the Cayenne variety, and since this soil in the field often shows considerable damage by pythiaceous fungi, we may conclude that the conditions of this soil favor the destruction of the plants or the activities of the pythiaceous fungi. In contrast to this soil stands the soil from a lower rainfall region, which seldom shows much damage in the field and did not show any appreciable differences in the mentioned test for Cayenne.

The fact that Pernambuco and lot 520 showed no appreciable differences in either soil seems to indicate that according to the one test made, these varieties are highly resistant. It is recognized that in the single root inoculation test the differences between these varieties and Cayenne were not so great. In the second water culture test, however, the percentage of rotted roots in Pernambuco was only 10.1 in contrast with 65.4 in Cayenne, a difference which seems comparable with the growth retardation test. If these data are adequate, they indicate that the rate of rotting measured in the single root inoculation tests reveals only a part of the actual differences in susceptibility which distinguish the varieties of pineapple.

SUMMARY

In a preliminary study of the relative susceptibilities of pineapple varieties to the root rot caused by *Nematosporangium rhizophthoron* Sideris, three different methods have been tried.

1. The rate of rotting of individual root tips has been determined for a large number of plants of 12 varieties and one F_1 hybrid population. Roots grown in soil in observation boxes were inoculated at their tips and then, after 48 hours, the length softened by the fungus was measured in millimeters. This method revealed statistically considerable differences between varieties. In Cayenne and lot 520 the mean lengths rotted were 18.08 ± 0.106 and 12.69 ± 0.124 . Wild Brazil, having an average rot of only 10.63 ± 0.398 , was the lowest in the series tested; however, the measurements of this variety were not high enough in number to give final evidence. The differences between the other varieties are less great, but still statistically significant.

2. By inoculating water cultures, the plant was considered as a unit and the mean percentage of roots rotted was determined for each variety. By

this method the percentage of infection was highest in Cayenne and lowest in Pernambuco, the other varieties being intermediate.

3. Plant growth during 6 months was measured in a third type of test using two types of sterilized soil as checks and also infested with a pure culture. The Cayenne variety, in a soil in which pythiaceous root rot frequently occurs in nature, showed considerable retardation of growth in the infested soil compared with the check. This retardation seems to be significant. In the other soil there were no differences in weight increase between the inoculated and uninoculated plants. Lot 520 and Pernambuco did not show any differences in weight increase in either soil. This appears to indicate resistance in these two varieties and susceptibility in Cayenne.

This study was made while the writer was an Exchange Fellow in Genetics at the Graduate School of Tropical Agriculture of the University of Hawaii. The writer takes great pleasure in expressing his thanks to Dr. R. N. Chapman for making this study possible by granting the exchange fellowship, and to Drs. J. L. Collins and M. B. Linford for valuable suggestions.

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A NEW *GLEICHENIOPSIS* FROM THE UPPER CRETACEOUS OF WESTERN GREENLAND¹

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While examining the collection of fossil plants from Western Greenland, made by Mr. Carl O. Erlanson in 1928, portions of small fertile *Gleicheniaceae* fronds were observed. Some of them were merely impressions, but others had a coating of the carbonized plant tissue still adhering to the fine dark gray shale. The latter fronds proved to be of much interest.

The carbonized material was carefully removed from the shale, placed in dilute nitric acid and potassium chlorate for a short time, and then treated with ammonium hydroxide. Each sorus or other part of the fronds was macerated and examined separately. Compact groups or masses of spores were readily liberated from each sorus, but it was almost impossible to separate the individual spores. Several different methods were employed but with no satisfactory results. A few small fragments of the epidermis from the rachises were also recovered. All of this macerated plant material was mounted on slides by the euparal method (Miner, 1932).

Tutin (1932) proposed the genus *Gleicheniopsis* for certain species included in *Gleichenites* which differed markedly from *Gleichenia* in having numerous small sporangia per sorus and very few spores; but the differences between the two genera are not sufficient, as is shown by the intermediacy of *Gleichenites Porsildii*. Nevertheless, since the distinction has been made, it seems best to continue the use of both generic names until more evidence is at hand. The age of *Gleicheniopsis* is given as Lower Cretaceous, but the specimen described here appears to belong higher up in the Cretaceous series. Heer (1882, 1883) divided the Cretaceous rocks of Western Greenland into three series: Kome or Lower Cretaceous, Atane or Cenomanian, and Patoot, the uppermost Cretaceous. Seward (1925, 1927) made a critical study and revision of this flora and concluded that a more thorough examination of the sections is necessary, because with the available facts it is difficult to correlate accurately the Cretaceous series of Greenland with other regions and also because this flora represents more fully than any other the early stages in the transitional period from an older Jurassic-Wealden vegetation to the later type that continued into the Tertiary.

The shale bearing this material came from Patoot and Atâ on the Nugsuaks

¹ Papers from the Department of Botany and Herbarium of the University of Michigan, No. 414.

Peninsula. The Patoot specimen was collected at 255 meters above sea level and the Atâ specimen from strata about one mile southeast of the Atâ delta at an altitude between 150 and 165 meters. It is apparent that this material belongs to Heer's Atane or Patoot series, and it also comes within the limits of the Upper Cretaceous of Western Greenland as given by White and Schuchert (1898).

Gleicheniopsis Erlansonii, sp. nov. Pinnae alternate; pinnules opposite, obliquely-opposite or alternate, 1–2 mm. wide, 1.2–2.5 mm. long, ovate or obliquely-ovate, confluent at the base by a slender common lamina, apex rounded, directed forward; sinuses narrowly acute, .1–.25 mm. in width at about the middle of the pinnules; sori 3–6 per pinnule, orbicular or nearly so, 500–900 μ in diameter (average about 650 μ), crowded; sporangia 16–40 per sorus, orbicular or nearly so, 70–150 μ in diameter (mean being about 105 μ); spore masses clavate to pyriform, 65–135 μ in width, 155–225 μ in length, the average size being about $100 \times 185 \mu$; spores few (probably 32) per sporangium, deltoid, 40–50 μ in diameter, with triradial clefts extending two-thirds or more of the distance to the periphery.—Patoot and Atâ on the Nugsuaks Peninsula, Western Greenland; Upper Cretaceous. Figures 1–6.

The data for the shape and size of the sporangia were taken from the sori of the various pinnules, as seen without macerating, such as the ones shown in figures 1 and 2. An annulus of a few unusually long indurated cells was observed on some of the sporangia but it was not well preserved, so that the exact number of cells could not be ascertained. Since the spores of each sporangium were always tightly compressed together into more or less the shape of the sporangium (see fig. 4 and 6), it was easy to determine the number of sporangia, per sorus, as each compact spore mass represents a single sporangium. It was almost impossible to separate the individual spores, so that the exact number in a sporangium could not be accurately determined, but it is estimated that the maximum number must be about 32. The characteristic epidermal cells of the rachis are shown in figure 3. Both specimens agree very closely on all measurements except that the Patoot specimen has 4, 5, or 6 sori on a pinnule and 16–30 sporangia in a sorus, whereas the Atâ specimen has 3, 4, 5, or 6 sori per pinnule and 16–40 sporangia per sorus. The most common number of sori on a pinnule is 4, and the others in the order of their occurrence are 5, 6, and 3.

The following table gives in summarized form the main diagnostic characters for the species in this genus:

Species	No. of sporangia per sorus	Diam. of sporangia	No. of spores in sporangium	Size of spores	No. of sori per pinnule
<i>Gleicheniopsis Erlansonii</i>	16–40	105 μ	32*	40–50 μ	3–6
<i>G. fecunda</i>	20–40	170 μ	18–26	50–60 μ	5–7
<i>G. Sewardii</i>	12–20	170 μ	32*	50 μ *	2–4

* Estimate.

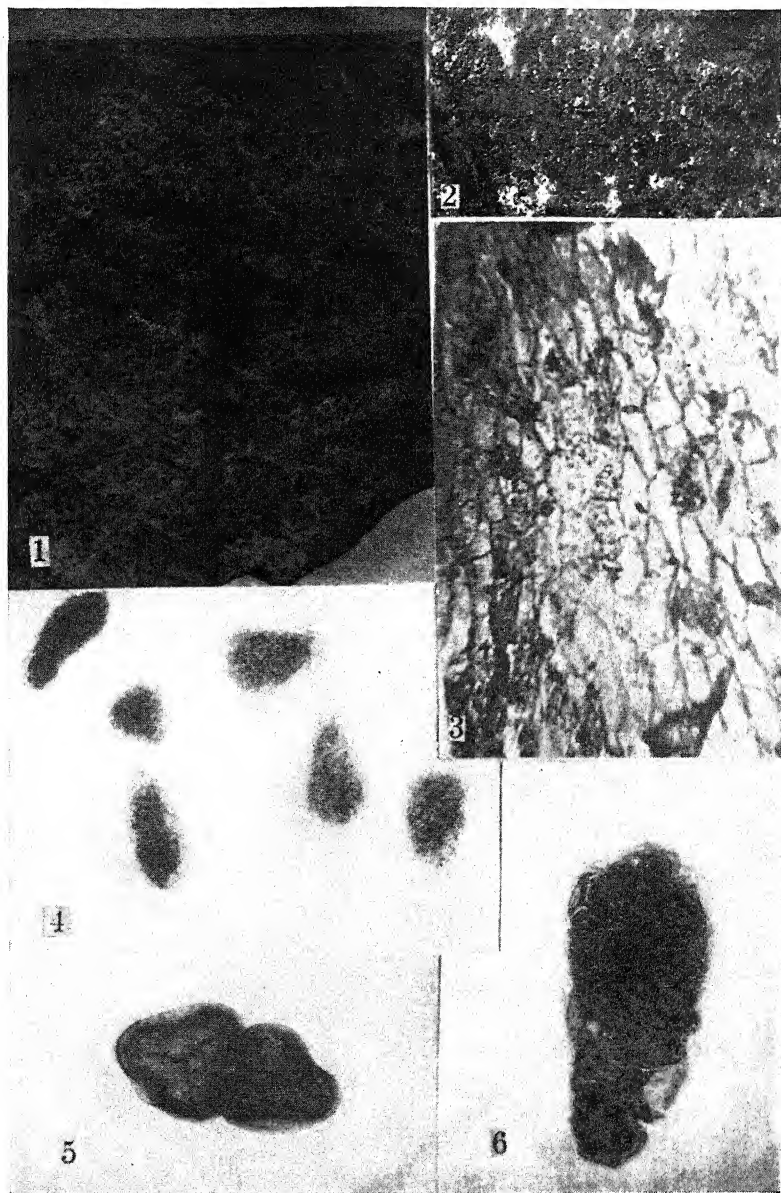


Fig. 1-6. *Gleicheniopsis Erlansonii*, sp. nov. Fig. 1. A portion of a frond showing the attachment of the pinnae to the rachis and the pinnules with their sori. $\times 27$. Atâ, Greenland. Fig. 2. Part of a pinna enlarged showing several pinnules with sori. $\times 5$. Patoot, Greenland. Fig. 3. Epidermal cells from the rachis. $\times 235$. Atâ, Greenland. Fig. 4. A group of six spore masses showing the variation in size and shape. $\times 106$. Patoot, Greenland. Fig. 5. Two characteristic spores separated from a spore mass, such as those shown in figures 4 and 6. $\times 340$. Patoot, Greenland. Fig. 6. A typical spore mass enlarged to show more clearly the compactness of the spores. $\times 240$. Atâ, Greenland.

A comparison of the above species shows that they are all closely related and that *G. Erlansonii* is intermediate in the most obvious characteristic, namely, the number of sori per pinnule, between *G. fecunda* and *G. Sewardii*. It is entirely possible that all three represent the range of variation in a single species, but this can only be proved by the examination and study of much more material. In general, there is every evidence that in the description of fossil florulae too great conservatism is as common as too great radicalism in the recognition of species. Certainly the preservation of the plant remains of any modern habitat would give many more species than are recognized in the average florula. Moreover, habitats removed as far in space from one another as many fossil localities that are compared would not have anywhere near the high proportion of species in common that paleobotanists usually find. With no desire to encumber the literature with unnecessary names, it seems desirable from a scientific standpoint to emphasize distinctions rather than supposed identities.

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GROWTH OF LIVERWORTS FROM KATMAI IN NITROGEN-FREE MEDIA¹

ROBERT F. GRIGGS AND DANIEL READY²

(Received for publication September 28, 1933)

A previous paper of this series (Griggs, 1933) has given an account of field work of the National Geographic Expedition of 1930 on the revegetation of the country covered by ash from the eruption of Katmai in 1912, wherein it was shown that the pioneer plants on pure ash deposits were leafy liverworts of the family Jungermanniaceae. The outstanding peculiarity of the ash upon which these plants were growing was the practical absence of nitrogen compounds. The obstacle to revegetation was, then, the difficulty of obtaining in available form the nitrogen necessary for the construction of protoplasm in conditions where the ordinary sources of such material almost totally fail.

The field work showed that where even very slight organic contamination occurred ordinary plants could grow on the ash, but that in the pure uncontaminated ash nothing except the liverworts obtained a foothold. Thus, secondary deposits of wind-blown or water-laid ash were occupied by plants of many species, but the flora of the undisturbed ash consisted of a pure stand of liverworts. These in places bore a copious crop of sporophytes. The liverwort beds, covering acre after acre of ground, developed a heavy carpet far denser and more extensive than any liverwort growth in normal habitats.

It was found, further, that the liverworts were the forerunners of other plants. Where they had occupied the ground longest, first mosses, then willows, and then grasses and other seed plants were coming in.

It was clear that an understanding of the nitrogen relations of these plants could not be gained from field observations alone but would require cultural studies under controlled conditions in the laboratory. The present paper presents the results obtained to date on this problem.

METHODS OF TRANSPORTING THE LIVERWORTS ALIVE FROM THE FIELD

The first requisite was to transport the liverworts alive from the wilderness of Katmai to the laboratory. This seemed in advance a formidable undertaking because of the apparent delicacy of the plants and of the six weeks' interval that must elapse between their collection on the ash flats and

¹ By means of a grant from the National Geographic Society, published out of the order determined by the date of receipt of the manuscript.

² Dr. Griggs did the cultural work in the botanical laboratory of George Washington University and wrote the paper. Mr. Ready provided the recrystallized salts used, did the analytical work, and was responsible throughout for the chemical basis of the results.

the beginning of their culture in Washington. Specimens were collected and carried in as many ways as could be thought of. Rather elaborate precautions were taken with some, but it was found that ordinary dried specimens gave as satisfactory cultural material as that carefully kept in the growing condition. Dried pieces of the liverwort layer promptly revived when placed on a bed of moist sand in a covered culture dish. Within an hour or two the growing tips began to expand and show green, and within a few days new branches had grown long enough to be transferred to other dishes. Dried material treated in this way sprouted freely after two, four, and six months but failed to grow after a twelve-month interval.

DESCRIPTION OF THE ORIGINAL SOURCE MATERIAL

The material thus used as the foundation of the cultures was a closely felted mat of densely branched liverworts of two species, which from their difference in size were designated in the field as the "big" liverwort and the "small" liverwort. They were determined by Dr. Alexander W. Evans, of Yale University, as *Lophozia bicrenata* (Schmid.) Dumort. and *Cephaloziella byssacea* (Roth.) Warnst., respectively. Both species sprouted and were recovered in culture. But the former remained almost stationary under the conditions supplied, while the latter grew vigorously. *Cephaloziella* alone, accordingly, was used for the work here reported.

Cephaloziella includes the smallest and most delicate species of the Jungermanniaceae. The stems are so tenuous that they have more of the aspect of coarse algae than of the mosses which most of the leafy liverworts resemble. Under a lens, however, they are readily placed in their proper order by the bifid scale-leaves characteristic of many genera in this group. Like other liverworts, they grow from an apical cell. But branches may originate at any point on the stem as old cells readily resume division and assume the character of new apical cells.

Though the liverworts as they grew on the ash constituted what foresters would call a pure stand in that no other plants of comparable size occurred among them, they were not of course a pure culture in the bacteriologist's sense. Numerous other organisms occurred as contaminations. These consisted of molds, bacteria, moss protonema, and a unicellular green alga which answered to the description of *Chlorococcum humicola* as given by G. M. Smith (1933). Blue-green algae were not present. Under field conditions the growth of these contaminants was suppressed and they were held to microscopic proportions. A study of the microorganisms, especially the bacteria, present with the liverworts has been made by N. R. Smith (1932).

CULTURE METHODS

Inasmuch as the prime requisite of further work was a plentiful supply of growing material, the first effort, disregarding the nitrogen problem for

the time being, was to stimulate growth by providing conditions as favorable as possible. The thought was that even though the liverworts might be able to fix nitrogen when starved to it, yet, like *Azotobacter*, they would grow better when supplied with plenty of combined nitrogen. The first cultures accordingly were made in saucers of good soil, sterilized, and upon volcanic ash or sand moistened with Shive's three-salt nutrient solution as recommended for wheat, known as "R5S2" with an osmotic concentration of 1.75 atmospheres. Other comparison cultures were, however, started on sand and ash moistened with a nitrogen-free modification of Shive's solution made by substituting calcium sulphate for calcium nitrate.³ To our surprise the liverworts did not grow so well in soil or in cultures supplied with nitrate or ammonia as in "nitrogen-free" media. Wherever abundant nitrogen was available, the moss protonema and algae, always present as contaminations, were stimulated to growth and the liverworts were soon overgrown. This result, repeatedly observed, abundantly confirmed the diagnosis of the situation in the field that the great development of liverworts on the ash flats is due to their ability to grow on media too poor in nitrogen for other organisms.

It should be pointed out, however, that the superiority of liverworts on "nitrogen-free" media in mixed cultures does not prove that nitrogen compounds are toxic to them. Later results detailed below, after the cultures had been freed from algae and mosses, did not bear out this idea. In pure cultures they did better if a little nitrogen was supplied than in its absence.

In addition to the first cultures in covered saucer-like dishes, others were made on agar plates and in liquid. The agar was prepared by using the N-free modification of Shive's solution as the liquid in which shredded agar was cooked. Experience showed that the liverworts grew best on very thin agar, just firm enough to hold its shape. There was in advance some question as to whether agar prepared in this way with the addition of nothing

³ Observations suggested that the liverworts did best in rather dilute media. The solution used for most of the work was made up to only two-fifths of the concentration recommended by Shive for wheat. Its composition was: KH_2PO_4 , 1.225 gm.; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 1.848 gm.; CaSO_4 (anhydrous), 0.340 gm.; iron as ferric phosphate, ferric chloride, or ferric citrate, trace; distilled water, 1000 cc.

To some of the cultures a small portion of Potomac River sand containing a large variety of minerals, from which all organic matter had been removed by heating to redness for several hours, was added on the theory that the liverwort might otherwise suffer from the lack of some of the elements, like zinc, boron, manganese, iodine, etc., which recent researches have shown to be necessary in infinitesimal amounts. Since, however, comparison cultures in liquid alone apparently did just as well as those provided with sand, this was omitted in later work.

Solution made up on the formula given above gave a reaction between pH 5 and 6 with a LaMotte soil "teskit"—i.e., the same as that found around rhizoids of the liverworts in the field. A more precise determination with a quinhydrone electrode made on the fluid poured off an old culture gave pH 5.08. Ashby's well-known solution as used for *Azotobacter* was also tried, but in this the liverworts soon died. The trouble was attributed to the alkaline reaction of this medium (pH 8).

but inorganic nitrogen-free salts would serve as a nitrogen-free medium. That is to say, it was uncertain whether the small amount of nitrogen present in the agar itself would be available to the plants. The agar used gave no reaction for nitrates or for ammonia and showed the faintest detectable trace of nitrites (see discussion of the sensitiveness of the tests below). But organic nitrogen was present to the extent of 2800 parts per million. The question of the availability of this organic nitrogen has been partially answered by planting various organisms in it. Moulds such as *Penicillium* and *Alternaria* persisted in it indefinitely, as we found to our sorrow, but could produce spores only feebly unless other organic matter such as weakened liverwort were present. Unicellular algae, such as *Chlorococcum*, which made no growth in liquid media made from the same salts as those used in the preparation of the agar multiplied extensively. Flowering plants chosen for the small size of their seeds gave varying results. *Antirrhinum* seedlings died as soon as the food stored in the seed was exhausted. *Agrostis alba*, however, grew for several months and began to stool out at the base before the agar dried out. Determination of the total nitrogen of this culture did not, however, definitely prove a greater quantity of nitrogen present than that in the original agar. These experiments were interpreted as indicating that the organic nitrogen of the agar, while unavailable to some plants, might be used by others.

As for the liverworts, they have continued growth on the "nitrogen-free" agar with unabated vigor for three years. There seems to be no limit to their growth except the drying out of the agar. By keeping them in moist chambers, individual cultures have now been kept going without change or addition for 21 months. Drying-up cultures can also sometimes be brought back by pouring over them fresh agar cooled nearly to the point of coagulation, about 40°C.

Although mats of liverworts similar to those under study were seen growing submerged in pools on the tundra, at Katmai, the first attempt to grow *Cephalosiella* in liquid media did not promise well. But when arrangements were made for frequent aeration, liquid cultures grew better than any others.

In the set-up finally adopted, both aerating flasks and agar Petri plates were used. Growth was faster in the flasks; but the plates were more convenient for examining the cultures, for checking up on contamination, and for making transfers.

PRECAUTIONS AGAINST ACCIDENTAL ACCESS OF NITROGEN COMPOUNDS

It was when preliminary results by the senior author indicated the possibility that liverworts could increase in media entirely free from combined nitrogen that Mr. Ready began active cooperation and undertook responsibility for the chemical side of the work.

Starting with Baker's analyzed reagents, the salts used for the culture so-

lutions, KH_2PO_4 , MgSO_4 , and CaSO_4 , were several times recrystallized and tested for nitrates, for ammonia, and for nitrites. In addition, the portions used in the preparation of each batch of culture solution and the distilled water were separately tested for nitrates, nitrites, and ammonia. *All these tests were negative.*

In order to assure ourselves as to the delicacy of the reactions employed, confirmatory tests were run on the sensitivity of the reagents used in estimating the various forms of nitrogen.

For nitrates, modified diphenylamine reagent was prepared according to the method of Withers and Ray (1910). While Withers and Ray claim positive findings in concentrations as low as one part in thirty-five million in the cold and one part in forty-four million after immersion in a water bath at 40° , the reagent employed by us with the most elaborate of precautions was found to be sensitive only to one part nitrogen as nitrate in five million of solution, or 0.2 mg. nitrogen as nitrate per liter of solution. Tests were run at fifteen-minute intervals each, in the cold and at 40° . We did not succeed in getting better results on the water bath than in the cold.

The sensitivity of the Nessler's reagent used by us to determine ammonia was found to be greater than one part of nitrogen as ammonia in ten million parts of solution, or to detect less than 0.1 mg. nitrogen as ammonia per liter.

Griess' reagent as used by us was found to possess a sensitivity of one part nitrite nitrogen per two hundred million, or a concentration of 0.005 mg. of nitrogen as nitrite per liter.

It should be pointed out that the reagents used for the culture media were tested in concentrated solutions, so that the limit of error in the dilute solutions made up for the plants was very far below these figures. Yet, since the sensitivity of the tests as applied to the distilled water could not be increased, it will be safer to consider the limit of error as given above.

The air which was bubbled through the cultures for aeration was first washed by passing successively through saturated sodium bicarbonate and concentrated sulphuric acid. The bicarbonate was expected to remove any of the oxides of nitrogen— NO_3 , NO_2 , etc.—which might be present. Sodium hydroxide would of course have been preferred for this purpose except that it would have removed also the carbon dioxide necessary for photosynthesis. The sulphuric acid was to remove any trace of ammonia. The air passing through the wash bottles was broken up into streams of fine bubbles by passing through a sintered glass filter.

As a further precaution blank flasks were placed between the wash bottles and the cultures. The thought was that any nitrogen compounds which might escape the wash bottles would go into solution in the blanks and be there detected.

Since the compressed air was bubbled through the cultures only intermittently, back draft from the air in the room induced by changes in temperature and barometric pressure constituted another possible source of con-

tamination. This was checked in three ways: The cultures were grown in a glass refrigerator case, kept between 60° and 70°F., where sudden temperature changes were minimized. The room in which they were grown was at a distance from any chemical operations which might involve the use of nitric acid or ammonia. Its air was tested by leaving tubes of Griess', Nessler's, and diphenylamine reagents open. At the end of twenty-four hours these reagents were unaffected. These tests were repeated from time to time. On one occasion the tubes were left exposed for three weeks, until after decomposition had set in, but at no time did they give any test for ammonia, nitrates, or nitrites.

Finally, the last flask in the series was heavily inoculated with one of the species of *Chlorella* used by Dr. F. B. Wann in his experiments on nitrogen fixation instead of with liverwort. It is known that *Chlorella* can grow in media containing very little combined nitrogen but that it cannot grow in the complete absence of such compounds. The *Chlorella* in this flask remained green for a time but it made no growth, although the liverworts in adjacent flasks of the same medium grew well. In this set-up any ammonia that might have been sucked in by back draft could reach the flasks of liverwort only after dissolving in the *Chlorella* culture, and re-evaporating and passing through a narrow tube into the liverwort cultures behind.

Since the *Chlorella* grew luxuriantly in the culture solution if a little ammonia or nitrate were added, its failure in the absence of such an addition proved that the amount of nitrogen available in the culture solution was at any rate below the minimum requirement of this alga.

In another series of cultures water buttercup, *Ranunculus delphinifolius*, was used in the trap-end of the line instead of *Chlorella*. This plant will thrive indefinitely in a covered jar of tap water where the nitrogen supply must be relatively low. But whereas the *Chlorella* remained green for weeks in the nitrogen-free medium without increasing, the buttercup showed ill effects almost at once and within a fortnight had died except for the terminal leaf, which looked pale and sickly and soon disappeared.

From time to time the flasks were opened for testing, and at the end of the experiments the medium was again tested with diphenylamine, Griess', and Nessler's reagents. *Throughout the work all tests for nitrates, nitrites, and ammonia in the reagents and culture media used were without exception negative.*

GROWTH OF THE CULTURES

The cultures were started from growing tips as small as could be conveniently picked off the source material with a pair of forceps under a binocular dissecting microscope. The filaments so transferred were 2-5 mm. long but on account of their slenderness were just at the edge of visibility to the naked eye. Sometimes the growing tip with its single growing point was transferred directly to the liquid medium. More often it was planted for a

time in a "nitrogen-free agar" plate where contaminations could be checked up before transfer to an aerating flask. When such a growing tip was transferred directly to one of the 500-cc. flasks employed, it was usually lost sight of for two or three weeks, or until it had grown enough to be found again in the relatively large body of liquid in which it was immersed.

Vigorous growth and dark green color continued in the bubbling flasks for periods varying from two to seven months. *Before growth ceased, the cultures increased to several hundred times their original size.*

After a shorter or longer period, however, the cultures turned pale and growth slowed down and gradually came to a standstill. It is, perhaps, not surprising that the cultures run out after two or three months in the same fluid. Most cultures of bacteria and other organisms slow down and have to be renewed at shorter intervals than that. In the case of water cultures of the higher plants with which these are most nearly comparable, standard procedure calls for renewal of the medium twice a week. But in a research like ours, where the introduction of very small quantities of impurities might vitiate our results, we felt it safer not to change the medium. Yet, considering the character of our problem, we felt that a satisfactory solution required that we succeed in finding the trouble and in growing our liverworts indefinitely in the same unchanged medium.

REPRODUCTION OF THE LIVERWORTS IN CULTURE

Sexual reproduction was not observed in any of the cultures. But vegetative propagation by gemmae occurred freely on the agar plates. The process was similar to that described as common in this group of plants by Campbell (1918) but occurred apparently in more than one way. Most frequently the cells of a growing tip would simply separate and start out each for itself. In other cases scale leaves were observed with some of their cells empty while unicellular gemmae were seen in the immediate vicinity. The inference was that the protoplasts had escaped from the old cell walls, but the actual escape of these bodies was never detected. According to Campbell, such a process, perhaps homologous to zoospore formation in the algae, is known in only one liverwort, *Aneura*.

The development of the gemmae, though quite irregular, is characteristic. There is first a series of divisions accompanied by considerable growth, which gives rise to a ball of cells resembling a colony of *Protococcus* but of very much larger cells. After a mass of 8-16 cells is produced in this way, division is localized and restricted to one plane, so that a filamentous protonema grows out from one side of the protococcoid mass. This may become slender and elongated or remain short and stubby. Branching is frequent; and the filamentous form is not strictly maintained, for irregular transverse divisions frequently occur. Very soon unicellular rhizoids are put out, arising especially from the basal protococcoid masses. After the protonemal filament

has attained a length of 10–20 cells, transverse divisions become more frequent, especially at the tip, and a more massive body is formed. Small appendages are soon seen on such tips, and in a short time they are established as ordinary leafy liverwort branches with apical cells from which further growth appears.

Regenerative protonemas similar to those arising from gemmae occur in old moribund cultures. One or two cells in an old stem frequently retain their vitality after their neighbors have perished and begin division, forming a protonema in all respects similar to that produced by a gemma.

CONTAMINATIONS

One factor in the inability of the cultures to continue growth indefinitely may have been the contamination by other organisms which were always present. In the beginning those consisted of molds, moss protonema, green algae, and bacteria. Blue-green algae never appeared except in a few open cultures and there only after an elementary class had studied blue-green algae in the same building. Inasmuch as the liverworts grow more slowly than the contaminants and seem too delicate to withstand the rough usage entailed in most disinfecting operations, the problem of securing pure cultures appears rather formidable.

In the beginning it was considered likely that the capacity of the liverworts to live in a nitrogen-free ash might be due to some symbiotic fungus or bacterium. Accordingly, no particular effort was made at first to eliminate foreign organisms from the cultures. Observation soon showed, however, that the fungi, at any rate, were deleterious, and many cultures were lost before molds were eliminated.

The only method readily available for getting rid of contaminations was to pick out apparently clean filaments and transfer these to new cultures. The moss protonema and the algae gave even more trouble than the fungi. Both grew faster than the liverworts, and as the infection showed up in culture after culture it seemed as though we should never succeed in getting rid of them. After three months, however, the last protonema was left behind, but the algae continued to give trouble. As the form involved, *Chlorococcum*, produced zoospores freely, it repeatedly broke out and invaded clean cultures from adjacent ones which carried so little contamination that it had been overlooked. But finally, it also was eliminated.

The algae also were decidedly injurious to the liverworts. It was observed that cultures heavily infested with algae turned pale and reached their limits much more quickly than those that were clean.

Since the culture solutions contained only inorganic salts with no source of energy, ordinary bacteria gave no trouble. Bacterial contamination was for the most part limited to one organism, *Bacillus radiobacter*, as determined by N. R. Smith (1932). This was, however, constantly present, and no

means has yet been found to destroy it without killing the liverworts.⁴ *Bacillus radiobacter* is an organism allied to the nitrogen-fixing root-nodule bacteria, to *Aerobacter aerogenes*, and to *Phytomonas tumefaciens*. Although able to grow in the usual nitrogen-free media, it is not able to fix nitrogen in appreciable amounts. Its growth consists largely of slime which is carbohydrate in nature. *B. radiobacter* is generally present in soil around plant roots, but its rôle in the economy of nature is not understood. It is likewise the most constant and abundant microbial associate of the liverworts in the field, as determined by studies of the material brought back from Katmai. Smith studied the nitrogen-fixing capacity of this strain by standard culture methods which were run parallel to cultures of *Azotobacter* and *B. megatherium*. The latter grows vigorously in the presence of available nitrogen but does not develop in its absence. The tests showed that the usual amount of nitrogen was fixed by *Azotobacter*, and that neither *B. radiobacter* nor *B. megatherium* increased the nitrogen content of the original solution.

CHEMICAL ANALYSIS

After the cultures had apparently reached the limit of their growth, analyses were made to determine their nitrogen content.

First, a single flask, though too small a sample to give reliable results with the method, was taken for a preliminary run to see what might be expected, and then the contents of 33 flasks were consolidated to make a specimen large enough for more dependable results.

The liverworts in these 34 flasks were derived from the subdivision of the progeny of two single growing points picked from pieces of resuscitated liverwort layer on November 30, 1930. They were analyzed on August 5 and 6, 1931. One of these growing tips, from which five flasks were derived, was grown for 37 days on agar made with nitrogen-bearing Shive's solution before transfer to nitrogen-free media. The other, from which 29 flasks came, never had access to more combined nitrogen than could be obtained from the "nitrogen-free" agar upon which its transplants grew for varying periods. In all cases, however, practically the entire growth had occurred in the flasks of liquid as nearly free from combined nitrogen as could be prepared.

When the experiment was terminated, the cultures were spherical green masses averaging about 25 mm. in diameter in a clear colorless solution which gave no evidence of organic matter (fig. 1). When air-dried, they weighed a total of 0.2488 gm. Upon heating in an oven at 105°C. they lost 6.2 per cent more moisture, shrinking to 0.2333 gm., which was, then, the dry weight upon which the percentages were computed.

⁴ Since this was written the liverwort has been isolated in pure culture by Dr. F. E. Allison of the Fixed Nitrogen Laboratory. In pure cultures growth is somewhat better than in mixed, but otherwise no essential difference appears as yet.

The culture solution, after concentration by boiling down with the addition of sulfuric acid to prevent the volatilization of any ammonia, gave no test for nitrates with diphenylamine, for nitrites with Griess' reagent, or for ammonia with Nessler's reagent. Nor did it yield any organic nitrogen by the Winkler boric acid modification of the Kjeldahl method.

The minuteness of the preliminary sample precluded the assignment of any very definite value to the results obtained. Organic nitrogen of the same order of magnitude as in the larger samples was, however, recovered.

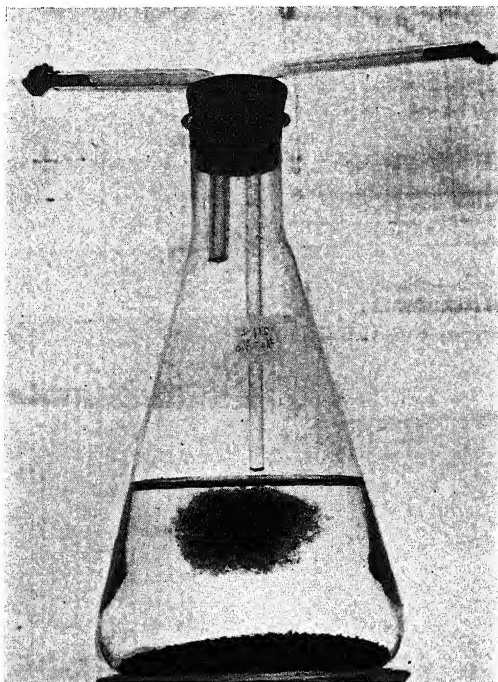


Fig. 1. One of the cultures just before analysis. Starting from a filament as small as could be picked out, the culture increased several hundredfold in the medium, which was as nearly free from combined nitrogen as could be prepared.

The larger consolidated sample was divided into two parts to check against accidental errors or variations in the material. These weighed dry 0.0938 gm. and 0.1395 gm. and gave 0.36 mg. and 0.52 mg. of nitrogen, respectively, —averaging therefore in round numbers 3800 parts per million of nitrogen as organic N.

DISCUSSION OF CHEMICAL RESULTS

Work such as this is necessarily compared with cultures of nitrogen-fixing bacteria and blue-green algae, which are the only other organisms known to grow extensively in nitrogen-free media. It should not be forgotten, how-

ever, that in one important respect the culture medium used is not at all comparable with those employed in other work on nitrogen fixation. Both *Azotobacter* and alga cultures are richly supplied with sugar or other carbon compound as a source of energy, but the liverworts had nothing except inorganic salts. It is the sugar supplied to the bacteria which makes possible the very rapid growth observed in bacterial cultures and the building up of considerable amounts of nitrogen compounds in short periods, whereas the liverworts must grow very slowly, seemingly taking their whole sustenance from inorganic materials.

On the other hand, such abundance of carbohydrate nutrient as is supplied to bacterial cultures does not occur in nature in the absence of combined nitrogen, and hence, as Bonazzi (1924) has pointed out, the rôle of *Azotobacter* and similar free living nitrogen fixers in the economy of nature is problematical.

The ability of the liverworts to increase several hundredfold in cultures where every possible precaution had been taken to prevent the access of nitrogen in forms other than the uncombined gas suggests that they may be able to build their protoplasm by fixing atmospheric nitrogen. Yet the analyses do not definitely prove that this occurred. The tests for ammonia and for nitrites are probably sufficiently delicate to prove that these compounds were not present in the medium in more than negligible quantities. But this is not true of the test for nitrate-nitrogen, which, being sensitive only to about 0.2 of one part per million, admits of an error too large to permit definite conclusions in our problem.

Calculation shows that if the liverworts were able to utilize the very last traces of nitrates it would be possible for them to secure from the amount of culture solution employed, even though it carried no more than one-fifth of one part per million of nitrate nitrogen, as much nitrogen as was recovered in the final analysis. The analyses therefore make it appear possible that the liverworts in our cultures were able to subsist for a while on the impurities in the best "nitrogen-free" medium that could be prepared and that it was the exhaustion of this supply of nitrogen that made them finally turn pale and run out.

CULTURAL TESTS OF THE CHEMICAL RESULTS

Reasoning that the limitation of growth observed in the cultures might be due to nitrogen starvation ensuing upon the exhaustion of hypothetical impurities in the medium, it was concluded that the addition of nitrogen might rejuvenate them.

Accordingly, tests were made in another series of cultures which had been growing in aerating flasks for 100 days and were still green and vigorous. To two of these flasks 2.5 mg. of nitrogen in the form of $(\text{NH}_4)_2\text{SO}_4$ were added. As there were about 250 cc. of liquid, this gave a nitrogen concentration of approximately 10 ppm. Two weeks later the nitrogen cultures

seemed a trifle darker than the others. At the end of three weeks they were definitely darker. After 50 days they were twice as large and growing vigorously while the checks, now 150 days old, had stopped growing and begun to lose color. The nitrogen cultures continued to do well and at the end of 204 days were several times larger than the checks.

The ability of the liverworts thus to continue growth indefinitely in the same unchanged medium provided ammonia were supplied seemed to dispose of the fear, previously entertained, that the accumulation of toxic waste products from their metabolism might be the limiting factor inhibiting further growth.

Having now demonstrated that growth could continue in an ammonia-bearing medium, it was of interest to ascertain the minimum concentration of ammonia necessary to maintain vigor. Accordingly, ammonium sulfate in varying amounts was added to the rest of the cultures of the series. This was at the end of the 150 days, when the cultures had reached their limits but had not begun to deteriorate.

The cultures thus treated, in contradistinction to all others previously dealt with, were now presumably almost absolutely free from combined nitrogen. For after being made up in the beginning as nearly free from nitrogen as could be accomplished by chemical means and as certified by chemical tests, they had supported the liverworts for five months. During this time if the plants were living on undetectable nitrogenous impurities, all traces of available nitrogen must have been removed, as evidenced by the cessation of growth. Here then was the first opportunity to determine the nitrogen requirements of the plants under conditions where all available nitrogen compounds had been rigorously excluded.

There were 16 flasks in the series besides the alga flasks at the end and the uninoculated blank at the front. Two of them had already received 10 ppm. of N, as described above. Two others, the best two, were left without addition. Of the others, two received 0.01 mg. of N, making the concentration 0.04 ppm.; one received 0.05 mg., or 0.2 ppm.; five received 0.1 mg., or 0.4 ppm.; one received 0.02 mg., or 0.8 ppm.; one received 0.5 mg., or 2.0 ppm.; and one received 1.0 mg., or 4.0 ppm. The two which received more than 1 ppm. were definitely benefited, turned green, and resumed growth. Those which received 0.8 and 0.4 ppm. gave slight and transitory signs of improvement. Where less than that amount was added, no response occurred.

These results might seem to make it permissible to set the minimum ammonia concentration required by the liverwort around 1.0 ppm. If this were done and this amount were subtracted from the hypothetical maximum impurities present in the culture jars, then nitrogen fixation would appear more probable.

This, however, does not seem to us to be a safe or conservative conclusion, particularly in view of later work with these same cultures. After they had

run their course with the nitrogen additions given above, the old liverworts were removed and the culture medium was boiled and reinoculated. This time practically no growth occurred. Reinoculation was tried a second time; again no growth occurred. Meanwhile, one of the cultures to which ammonia had been originally added, with still further additions from time to time as growth seemed to flag, continued to increase and still remains green at the end of three years.

SUMMARY

The cultures decisively confirm the opinion reached from field observation that the liverworts, especially *Cephalosiella*, can grow on media lower in combined nitrogen than any ordinary plants. Specifically in the cultures they increase many hundredfold in media that allow no growth to *Ranunculus delphinifolius* or to algae of the genus *Chlorella*. This capacity explains the extensive liverwort carpets on the ash flats of Katmai.

The inability of the liverworts to compete with mosses and algae in nitrogen-bearing media explains their comparatively scanty development in more favorable habitats.

While it is not impossible that the liverworts may be able to build their protoplasm by the fixation of atmospheric nitrogen, such ability has not been demonstrated by the experiments.

The more conservative conclusion is that their growth in "nitrogen-free" media was accomplished by the utilization of nitrogenous impurities present in concentrations below the thresholds of the tests available. This conclusion seems substantiated by the failure of repeated reinoculations of old cultures in which the liverworts had run out, whereas the addition of ammoniacal nitrogen to the extent of 1 ppm. served to rejuvenate the plants in otherwise untouched cultures.

In view of the very slow growth of the liverworts it may be possible for them to subsist on ammonia from rainfall, even though this has been proven (Griggs, 1933) to be much less in the Katmai region than in civilized countries.

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INHERITANCE OF RESISTANCE TO LOOSE AND COVERED SMUT IN HYBRIDS OF BLACK MESDAG WITH HULL-LESS, SILVERMINE, AND EARLY CHAMPION OATS¹

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The present paper is concerned with the results obtained in an investigation of the inheritance of resistance to loose (*Ustilago Avenae* (Pers.) Jens.) and covered (*U. levis* (K. & S.) Magn.) smut in hybrids of Black Mesdag with Hull-less, Silvermine, and Early Champion oats. The variety Black Mesdag has consistently shown complete resistance to these two races of the oat smuts. It has been grown along with the hybrids in all of the experiments and, as recorded in table 1, 741 plants were inoculated with loose smut and 868 with the covered, no infected plants being observed in either series. This variety has also shown resistance to nearly all the races of both loose and covered smut. On the basis of extensive data, 9 distinct races of loose smut and 5 races of covered smut have been differentiated, and not one of these has successfully attacked Black Mesdag (Reed, 1929). It is, however, susceptible to a newly discovered race of covered smut (Reed, 1932c; Reed and Stanton, 1932), and this fact emphasizes the importance of using known races of smut in studies of the inheritance of resistance in oat hybrids.

In contrast to Black Mesdag, the Hull-less, Silvermine, and Early Champion varieties are highly susceptible. They have been inoculated with the loose and covered smut and grown along with the hybrids each year, the data being recorded in table 1. With the loose smut, Hull-less has given 95.2 per cent infection, Silvermine 72.3 per cent, and Early Champion 95.7 per cent. With the covered smut, Hull-less has given 73.5 per cent infection, Silvermine 74.2 per cent, and Early Champion 87.8 per cent. In one or more experiments, all of these varieties gave 100 per cent infection; in individual rows, however, a few normal plants occurred. The lowest percentage of infection obtained at any time with these varieties was 52.1 per cent. It is evident that these three varieties—Hull-less, Silvermine, and Early Champion—are highly susceptible to the Missouri races of loose and covered smut, and thus afford

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a striking contrast to the behavior of Black Mesdag. They have been crossed with Black Mesdag, and the inheritance of resistance to both smuts in the second, third, and fourth generations has been studied.

I have previously published data on one cross of Hull-less \times Black Mesdag (Hybrid 2) in which the second, third, and fourth generations were inoculated with the loose smut (Reed, 1925). In a later publication the results for the second and third generations of four other crosses of these two varieties (Hybrids 10-13), and also for the second generation of two crosses of Silvermine \times Black Mesdag (Hybrids 17 and 18) have been recorded (Reed, 1928). Further data on the fourth generation of Hybrid 2, on the second and third generations of Hybrids 10-13, and on the second generation of Hybrids 17 and 18 have been obtained, as well as extensive data on the third and fourth generations of Hybrids 17 and 18, and on the second, third, and fourth generations of Hybrids 33-36, the crosses of Early Champion with Black Mesdag. For the sake of completeness, the older data have been incorporated in the tables and summaries in the present account. A brief statement of many of these results has been published (Reed, 1932d), and annual progress reports have been made in the Brooklyn Botanic Garden Record. The methods employed have been fully described elsewhere (Reed, 1932b).

EXPERIMENTAL RESULTS WITH THE F_2 GENERATION

Three sets of second generation plants of all the hybrids, with one exception, were grown. One set was inoculated with the loose smut, a second set with the covered smut, and a third set was grown without any inoculation. In the experiments with Hybrid 2, Hull-less \times Black Mesdag, only two sets of F_2 plants were grown, one inoculated with the loose smut, and the other uninoculated.

Hull-less \times Black Mesdag. All the data obtained for the second generation with the five hybrids of Hull-less \times Black Mesdag are recorded in table 1. Altogether, there were 738 F_2 plants of the five hybrids inoculated with the loose smut, and 170 (23.0 per cent) were infected. Hybrid 12 gave the lowest percentage of infection (20.2 per cent), and Hybrid 11 the highest (25.7 per cent). In the parallel series with the covered smut, 488 F_2 plants belonging to Hybrids 10-13 were inoculated, and 92 (18.8 per cent) were infected. The lowest percentage of infection (12.5 per cent) was obtained with Hybrid 12, and the highest (22.8 per cent) with Hybrid 13.

Silvermine \times Black Mesdag. There were 124 F_2 plants of Hybrids 17 and 18 inoculated with the loose smut, and 25 (20.1 per cent) were infected. In the parallel series with the covered smut, there were 115 inoculated F_2 plants, and 22 (19.1 per cent) were infected.

Early Champion \times Black Mesdag. There were 278 F_2 plants of Hybrids 33-36 inoculated with the loose smut, and 62 (22.3 per cent) were infected, the percentages varying from 18.9 in Hybrid 34 to 29.7 in Hybrid 33.

In the series with the covered smut, 317 F_2 plants were inoculated and 57 (17.9 per cent) were infected. The lowest percentage was obtained with Hybrid 36, in which only 7 out of 74 plants (9.4 per cent) were smutted; the highest percentage (26.8 per cent) was obtained in Hybrid 35.

The combined results of all of these crosses indicate a similarity in their behavior. The number of second generation plants grown is fairly large, and the results with all three sets of hybrids suggest that resistance to both smuts is dominant, and that segregation occurs on the basis of a three-to-one ratio.

EXPERIMENTAL RESULTS WITH THE F_3 GENERATION

Since there were three sets of F_2 plants, the F_3 progenies grown from them were divided into three distinct groups: (1) Progenies descended from F_2 plants which had been inoculated with the loose smut; (2) progenies descended from F_2 plants which had been inoculated with the covered smut; and (3) progenies descended from uninoculated F_2 plants.

The data for all the F_3 progenies are summarized in table 2. The results for Hybrids 2, and 10-13, Hull-less \times Black Mesdag, have already been published in detail (Reed, 1925, 1928). The data for the progenies of Silver-

TABLE 1. Results with inoculated F_2 plants of crosses between susceptible oat varieties and Black Mesdag

	Inoculated with <i>Ustilago Avenae</i>			Inoculated with <i>Ustilago levis</i>		
	No. plants	No. inf.	Per cent inf.	No. plants	No. inf.	Per cent inf.
Hull-less \times Black Mesdag						
Hybrid no. 2	82	21	25.6	—	—	—
10	168	38	22.6	132	25	18.9
11	171	44	25.7	125	25	20.0
12	143	29	20.2	104	13	12.5
13	174	38	21.8	127	29	22.8
	738	170	23.0	488	92	18.8
Silvermine \times Black Mesdag						
Hybrid no. 17	76	19	25.0	72	12	16.6
18	48	6	12.5	43	10	23.8
	124	25	20.1	115	22	19.1
Early Champion \times Black Mesdag						
Hybrid no. 33	74	22	29.7	74	14	18.9
34	79	15	18.9	102	18	17.6
35	80	16	20.0	67	18	26.8
36	45	9	20.0	74	7	9.4
	278	62	22.3	317	57	17.9
Reaction of parental varieties						
Black Mesdag	741	0	0	868	0	0
Hull-less	212	202	95.2	231	170	73.5
Silvermine	430	311	72.3	481	357	74.2
Early Champion	466	446	95.7	594	522	87.8

TABLE 2. Summary of results with the F_2 progenies of hybrids of Hull-less \times Black Mesdag, Silvermine \times Black Mesdag, and Early Champion \times Black Mesdag

		Reaction of F ₂ progenies to both <i>Ustilago Avenae</i> and <i>U. levis</i>			
		Similar			Dis- similar
		Re- sistant	Seg- regating	Sus- ceptible	
		Total			
1. F ₂ plants inoculated with <i>U. Avenae</i>					
Hull-less × Black Mesdag					
Hybrids 10-13	286	98	183	0	5
Silvermine × Black Mesdag					
Hybrids 17, 18	78	30	41	3	4
Early Champion × Black Mesdag					
Hybrids 33-36	95	27	66	1	1
2. F ₂ plants inoculated with <i>U. levis</i>					
Hull-less × Black Mesdag					
Hybrids 10-13	98	25	65	2	6
Silvermine × Black Mesdag					
Hybrids 17, 18	90	31	50	1	8
Early Champion × Black Mesdag					
Hybrids 33-36	142	36	93	10	3
3. F ₂ plants not inoculated					
Hull-less × Black Mesdag					
Hybrids 10-13	194	56	91	42	5
Silvermine × Black Mesdag					
Hybrids 17, 18	69	19	31	11	8
Early Champion × Black Mesdag					
Hybrids 33-36	72	17	33	17	5

mine \times Black Mesdag and Early Champion \times Black Mesdag are arranged in tables 3 and 4. The families are not listed separately, but they are grouped in percentage classes. The results secured with the loose smut are arranged independently from those obtained with the covered smut.

On the basis of the behavior of the third generation progenies, they were classified as resistant, segregating, or susceptible. The resistant progenies were those in which no infected plants were found; the segregating progenies included all those in which the percentage of infected individuals was less than 50 per cent; the susceptible progenies included all those in which more than 50 per cent of the plants were infected. In most of the susceptible progenies a high percentage of infection, sometimes 100 per cent, was obtained.

The third generation progenies may also be grouped on the basis of their reaction to the loose and covered smut. One of the most striking features of the results is the fact that a given third generation progeny behaved in a similar manner to both smuts. If it was resistant to one smut, it was also resistant to the other; if it was segregating to the one smut, it also segregated to the other; and finally, if it was susceptible to one smut, it was susceptible to the other. Some progenies, however, proved to be dissimilar in their reac-

TABLE 3. Data for the F_2 progenies of Hybrids 17, 18, Silvermine \times Black Mesdag

Class center	F_2 progenies inoculated with <i>Ustilago Avenae</i>				F_2 progenies inoculated with <i>Ustilago levis</i>			
	No. prog.	No. plants	No. inf.	Per cent inf.	No. prog.	No. plants	No. inf.	Per cent inf.
F_2 plants inoculated with <i>Ustilago Avenae</i>								
0	32	750	0	0	30	880	0	0
5	4	93	7	7.5	13	373	30	8.0
15	16	354	58	16.3	24	729	115	15.7
25	15	411	103	25.0	6	198	50	25.2
35	3	78	27	34.6	1	34	11	32.3
45	3	56	26	46.4	1	34	14	41.1
55	0	0	0	0	1	19	11	57.8
65	0	0	0	0	0	0	0	0
75	3	59	45	76.2	2	49	36	73.4
85	0	0	0	0	0	0	0	0
95	2	20	19	95.0	0	0	0	0
F_2 plants inoculated with <i>Ustilago levis</i>								
0	33	962	0	0	36	1249	0	0
5	6	138	10	7.2	25	1047	56	5.3
15	25	785	132	16.8	19	651	93	14.2
25	17	478	116	24.2	6	219	48	21.9
35	6	206	67	32.5	3	106	35	33.0
45	1	27	11	40.7	0	0	0	0
55	0	0	0	0	0	0	0	0
65	1	41	26	63.4	1	24	15	62.5
75	0	0	0	0	0	0	0	0
85	0	0	0	0	0	0	0	0
95	1	17	16	94.1	0	0	0	0
F_2 plants not inoculated								
0	20	435	0	0	22	562	0	0
5	4	93	7	7.5	15	467	25	5.3
15	21	521	77	14.7	13	352	51	14.4
25	6	130	29	22.3	4	128	32	25.0
35	4	102	38	37.2	0	0	0	0
45	0	0	0	0	4	139	61	43.8
55	0	0	0	0	3	99	56	56.5
65	3	100	69	69.0	3	111	71	63.9
75	5	193	148	76.6	0	0	0	0
85	3	102	90	88.2	3	112	94	83.0
95	3	68	66	97.2	2	58	56	96.5

tion. Several of these were retested and, on the basis of the second test, were classified as similar in their behavior to both smuts. With many of these a second test has not been made, while with others the results of the second test corresponded with those of the first.

All the data obtained for the third generation progenies are summarized in table 2. The progenies are grouped on the basis of their behavior to both loose and covered smuts, as determined by the original experiments and also the repeated tests.

TABLE 4. *Data for the F₃ progenies of Hybrids 33-36, Early Champion × Black Mesdag*

Class center	F ₃ progenies inoculated with <i>Ustilago Avenae</i>				F ₃ progenies inoculated with <i>Ustilago levis</i>			
	No. prog.	No. plants	No. inf.	Per cent inf.	No. prog.	No. plants	No. inf.	Per cent inf.
F ₂ plants inoculated with <i>Ustilago Avenae</i>								
0	27	669	0	0	27	735	0	0
5	8	170	15	8.8	16	460	31	6.7
15	20	481	73	15.1	28	685	104	15.1
25	27	658	160	24.3	16	432	103	23.8
35	10	222	77	34.6	7	196	66	33.6
45	1	15	7	46.6	0	0	0	0
55	0	0	0	0	0	0	0	0
65	2	72	50	69.4	1	42	27	64.2
75	0	0	0	0	0	0	0	0
85	0	0	0	0	0	0	0	0
95	0	0	0	0	0	0	0	0
F ₂ plants inoculated with <i>Ustilago levis</i>								
0	36	841	0	0	38	1049	0	0
5	3	58	5	8.6	17	532	30	5.6
15	30	753	128	16.9	35	1079	163	15.1
25	40	1137	202	25.6	34	953	236	24.7
35	20	520	181	34.8	8	240	82	34.1
45	2	57	24	42.1	0	0	0	0
55	0	0	0	0	1	25	13	52.0
65	0	0	0	0	2	47	31	65.9
75	1	24	18	75.0	4	91	68	74.7
85	3	65	55	84.6	3	91	78	85.7
95	7	182	176	96.7	0	0	0	0
F ₂ plants not inoculated								
0	17	376	0	0	18	424	0	0
5	2	45	4	8.8	11	308	16	5.1
15	9	225	37	16.4	15	395	62	15.6
25	13	379	100	26.3	7	186	40	21.5
35	8	179	60	33.5	4	158	59	37.3
45	1	23	10	43.4	6	251	118	47.0
55	1	23	12	52.1	4	176	99	56.2
65	0	0	0	0	1	44	30	68.1
75	2	88	70	79.5	4	175	128	73.1
85	8	317	272	85.8	1	41	33	80.4
95	11	471	445	94.4	1	21	20	95.2

F₃ progenies descended from F₂ plants which had been inoculated with loose smut

Hull-less × Black Mesdag. According to the data already published (Reed, 1925) for Hybrid 2, there were 42 F₃ progenies inoculated with the loose smut, and 12 of these were resistant and 30 segregating. None of these progenies were inoculated with the covered smut. There were 286 F₃ progenies of Hybrids 11-13 which descended from F₂ plants inoculated with the

loose smut, and of these, 98 were resistant and 183 segregating to both loose and covered smut. No families susceptible to both smuts were found. There were, however, 5 families which showed a dissimilar reaction.

Silvermine \times *Black Mesdag*. A total of 78 progenies of Hybrids 17 and 18 descended from F_2 plants inoculated with loose smut were grown. Of these, 30 gave no infection; 41 were classified as segregating; 3 proved to be susceptible; and 4 showed a different behavior to the two smuts. The 3 susceptible progenies gave 77.2 to 93.2 per cent infection with the loose smut and 57.8 to 78.5 per cent infection with the covered.

Early Champion \times *Black Mesdag*. There were 95 progenies of Hybrids 33, 34, and 35 belonging to this group. Of these progenies, 27 gave no infection; 66 gave percentages varying between 6.2 and 37.5 per cent infection with the loose smut, and from 4.1 to 34.1 per cent with the covered smut. One progeny was classified as susceptible to both smuts, since 70.0 per cent of the plants were infected with the loose smut and 64.2 per cent with the covered. One progeny showed a dissimilar reaction.

Altogether, 459 progenies belonging to the three sets of hybrids were grown, and 449 behaved in a similar manner to loose and covered smut. There were 4 progenies classified as susceptible to both loose and covered smut. The inoculation of the F_2 plant with loose smut should have the effect of killing off all, or practically all, of the individuals susceptible to it; consequently, there should be no susceptible progenies in the following generation unless a susceptible F_2 plant escaped. It is an interesting fact that these 4 progenies proved to be susceptible to both loose and covered smut.

The results for the second generation indicate that smut resistance is inherited on the basis of a three-to-one ratio. Consequently, among these third generation progenies, we would expect one resistant to two segregating. The data obtained are in fairly close harmony with this expectation; with *Hull-less* \times *Black Mesdag* there were 98 resistant to 183 segregating; with *Silvermine* \times *Black Mesdag* there were 30 resistant to 41 segregating; and with *Early Champion* \times *Black Mesdag* there were 27 resistant to 66 segregating. On the basis of the number of resistant progenies found, there were not enough segregating families in the hybrids of *Hull-less* \times *Black Mesdag* and *Silvermine* \times *Black Mesdag*, and a few too many in the hybrids of *Early Champion* \times *Black Mesdag*.

F₃ progenies descended from F₂ plants which had been inoculated with covered smut

Hull-less \times *Black Mesdag*. There were grown 98 progenies of Hybrids 10-13 descended from F_2 plants inoculated with the covered smut, and 25 of these were resistant, 65 segregating, 2 susceptible, and 6 dissimilar in their reaction. The two susceptible progenies gave 100 per cent infection with the loose smut; one of these gave 61.1 per cent, and the other 82.3 per cent infection with the covered smut.

Silvermine \times *Black Mesdag*. There were grown 90 progenies of Hybrids 17 and 18 descended from F_2 plants inoculated with the covered smut, and 31 were resistant, 50 segregating, 1 susceptible, and 8 dissimilar in their reaction. The susceptible progeny gave 94.1 per cent infection with the loose smut and 62.5 per cent with the covered.

Early Champion \times *Black Mesdag*. There were 142 progenies of Hybrids 33-36 descended from F_2 plants inoculated with the covered smut, and 36 of these were resistant, 93 segregating, 10 susceptible, and 3 dissimilar in their behavior. The 10 susceptible progenies gave from 81.8 to 100 per cent infection with the loose smut and 52.0 to 87.5 per cent infection with the covered smut.

There is a remarkable parallelism in the reaction of the progenies of this group to both loose and covered smut. Altogether, there were 330 progenies of the three sets of hybrids grown, and 92 were resistant, 208 segregating, and 13 susceptible to both smuts. Only 17 progenies were classified as dissimilar in their reaction.

The inoculation of the F_2 plants with the covered smut should have the effect of killing off all of the individuals susceptible to it. Consequently, there should be no F_3 progenies giving a high percentage of infection, unless a susceptible F_2 plant escaped. Actually, there were 13 F_3 progenies belonging to these different hybrids classified as susceptible to the covered smut, indicating that a corresponding number of F_2 plants escaped infection; the same F_3 progenies were also susceptible to the loose smut. It will be noted that there is a higher proportion of susceptible F_3 progenies found in this group than in the group of F_3 progenies that descended from F_2 plants inoculated with loose smut; these results perhaps may be correlated with the data obtained on the F_2 generation where, in general, a somewhat lower percentage of infection was obtained with the covered smut as compared with the loose.

The remaining 300 F_3 progenies which reacted in the same fashion to loose and covered smut fall into the two groups resistant and segregating. With *Hull-less* \times *Black Mesdag*, there are 25 resistant to 65 segregating; with *Silvermine* \times *Black Mesdag*, 31 resistant to 50 segregating; and with *Early Champion* \times *Black Mesdag*, 36 resistant to 93 segregating. The results with all of these suggest the expected ratio of one resistant to two segregating. There are fewer resistant progenies in *Hull-less* \times *Black Mesdag* and *Early Champion* \times *Black Mesdag* than might be expected, while in *Silvermine* \times *Black Mesdag* there is an excess of this group of progenies.

F₃ progenies descended from uninoculated F₂ plants

Hull-less \times *Black Mesdag*. As previously published (Reed, 1925), there were grown 58 F_3 progenies of Hybrid 2, descended from uninoculated F_2 plants, which had been inoculated with the loose smut. 18 of these progenies were classified as resistant, 28 as segregating, and 12 as susceptible. None

of these progenies were inoculated with the covered smut. There were grown 194 F_3 progenies of Hybrids 10-13, descended from uninoculated F_2 plants, and 56 of these were resistant, 91 segregating, and 42 susceptible, to both loose and covered smut. In addition, there were 5 progenies which gave a dissimilar reaction.

Silvermine \times *Black Mesdag*. There were grown 69 F_3 progenies of Hybrids 17 and 18, descended from uninoculated F_2 plants, and 19 were classified as resistant, 31 as segregating, 11 as susceptible, and 8 as dissimilar in reaction.

Early Champion \times *Black Mesdag*. There were grown 72 F_3 progenies of Hybrids 33-36, descended from uninoculated F_2 plants, and 17 were classified as resistant, 33 as segregating, 17 as susceptible, and 5 as dissimilar in reaction. Among the susceptible progenies, there were 6 which, in the first test, gave a high percentage of infection with the loose smut and a much lower percentage with the covered and, on the basis of these results, they would be classified as dissimilar in reaction. A second test, however, was made with all of these 6 progenies, and more than 50 per cent infection was obtained in both the loose and covered smut series. However, if the results for both are combined, it will be found that while they all contain more than 50 per cent of infected plants in the loose smut series, they contain slightly less than that percentage in the covered smut series. There can, however, be no reasonable doubt about the susceptibility of these particular progenies to both smuts.

Since there was no inoculation of the F_2 plants from which these progenies were derived, the susceptible individuals, as well as the resistant, would be represented among the third generation progenies. On the basis of the behavior of the inoculated second generation plants, and also on the behavior of the third generation progenies of the two preceding groups, we would expect three classes of F_3 progenies—resistant, segregating, and susceptible. Altogether, there were grown 335 F_3 progenies from the uninoculated F_2 plants and of these, 92 were classified as resistant, 155 as segregating, and 70 as susceptible to both loose and covered smut. In addition, there were 18 dissimilar reacting progenies. The results suggest a segregation of these F_3 progenies on the basis of one resistant, two segregating, and one susceptible. There were a few too many resistant progenies in the hybrids of Hull-less \times Black Mesdag and Silvermine \times Black Mesdag.

THE F_3 PROGENIES DISSIMILAR IN THEIR BEHAVIOR TO LOOSE AND COVERED SMUT

Altogether, there were grown 1124 F_3 progenies of all the hybrids, and 45 were dissimilar in their behavior to loose and covered smut. The dissimilar F_3 progenies are distributed among all the hybrids, 16 of Hull-less \times Black Mesdag, 20 of Silvermine \times Black Mesdag, and 9 of Early Champion \times Black Mesdag. Further, they are found in all the three groups of

progenies, grouped upon the treatment of the F_2 plant. They may be classified as follows:

No. of progenies	<i>Ustilago Avenae</i>	<i>Ustilago levis</i>
9	Resistant	Segregating (1.5 to 33.3 per cent)
19	Segregating (2.7 to 33.3 per cent)	Resistant
15	Susceptible (57.8 to 100 per cent)	Segregating (13.9 to 42.1 per cent)
1	Segregating (40.0 per cent)	Susceptible (52.3 per cent)
1	Susceptible (52.1 per cent)	Resistant

It will be noted that 28 of these progenies were resistant to one smut and segregating to the other; 16 of the progenies were susceptible to one smut and segregating to the other; and only one was classified as susceptible to one smut and resistant to the other.

The first 28 progenies, with few exceptions, should probably be classified as segregating to both smuts. In a few cases, a single infected plant was found in one of the series and, when the second test was made, negative results were obtained with both smuts. For example, in the first experiment with Hybrid 18- F_3 -242, no infection was obtained with the loose smut, while one smutted plant was found in the covered smut series. A second test was made, and no infected plants were found in either series. Further, 12 F_4 progenies were grown, some plants being inoculated with the loose smut and others with the covered, and no infected plants were observed.

The next 15 progenies probably contain some which are segregating to both smuts, while others are susceptible to both. There are 5, however, which, on the basis of two trials, have given quite high percentages of infection with loose smut and comparatively low percentages with the covered. Further studies are being made on these progenies.

EXPERIMENTAL RESULTS WITH THE F_4 GENERATION

A large number of fourth generation progenies of the various hybrids was grown. Separate sets of seed were inoculated with loose and covered smut. For the most part, the F_4 progenies were derived from resistant F_3 families.

Hull-less \times *Black Mesdag*. In an earlier paper (1925) I have described some results with the fourth generation of Hybrid 2, inoculated with the loose smut. There were 54 F_4 progenies grown from 9 resistant F_3 families, and these contained 1025 plants, none of which were smutted. There were grown 12 F_4 progenies from 2 susceptible F_3 families; they contained 232 plants, of which 230 (99.1 per cent) were infected. Finally, there were grown 78 F_4 progenies from 13 segregating F_3 families; some of the F_4 progenies were classified as resistant, others as segregating, and others as susceptible, and frequently the group of F_4 progenies derived from the same F_3 family contained all three classes. The 78 F_4 progenies were classified as 19 resistant, 33 segregating, and 26 susceptible.

Additional data on 327 F_4 progenies grown from 16 resistant F_3 families

have been obtained. One set of seed was inoculated with loose smut and the other with the covered smut. The results were as follows:

4434 plants inoculated with loose smut, and 15 infected.

4309 plants inoculated with covered smut, and 4 infected.

The 15 plants infected with the loose smut were distributed in 6 progenies, descended from 2 different F_2 plants. The plants infected with the covered smut were found in 2 of these same progenies.

Silvermine \times *Black Mesdag*. There were grown 198 F_4 progenies of Hybrids 17 and 18, derived from 54 F_3 families. These were ultimately descended from F_2 plants, some of which had been inoculated with loose smut, others with the covered smut and, finally, others uninoculated. All of the F_3 progenies were pure resistant to both loose and covered smut, no infected plants being observed in any of them. A total of 2843 F_4 plants were inoculated with the loose smut and 4 were infected. In the covered smut series, there were 3758 F_4 plants, and 5 were smutted. The 4 plants infected with the loose smut, and 3 of those infected with the covered smut, were found in the same two F_4 progenies; the remaining 2 plants infected with the covered smut were found in another F_4 progeny.

Early Champion \times *Black Mesdag*. There were grown 194 F_4 progenies of Hybrids 33-36. These were descended from 42 F_3 families, none of which had given any infection with either loose or covered smut. In the loose smut series, there were 3393 F_4 plants inoculated and 6 were infected. In the covered smut series, there were 3632 plants inoculated and 3 infected. These infected plants were found in two of the F_3 progenies. Hybrid 36- F_3 -121 was represented by 5 F_4 progenies, of which 4 were resistant and 1 contained 3 smutted plants in the loose smut series, while all the progenies were resistant to the covered smut. Hybrid 34- F_3 -207 was represented by 3 progenies, one of which contained 3 smutted plants in both the loose and covered smut series.

No infected plants were found in either series of the third generation progenies of Hybrid 36- F_3 -118. This hybrid was represented by 5 F_4 progenies; 4 of these segregated in both series, and 1 was susceptible. The susceptible progeny contained 22 plants inoculated with the loose smut, all of which were infected, and 25 plants inoculated with the covered smut, of which 17 (68.0 per cent) were infected. It is probable that the third generation progenies escaped infection in both the loose and covered smut series. Among the dissimilar F_3 progenies in the various hybrids, we find a few which show no infection with one smut, while some plants are attacked by the other. On a retest of such progenies, smutted plants are frequently found in both series. It is not improbable, then, that in such a large series of F_3 progenies, we might find one or more which genetically contains individuals susceptible to both smuts, and yet shows no infected plants in either series. Appar-

ently, only one F_3 progeny, out of all those tested, seems to have escaped infection in both series.

The F_4 progenies of all the hybrids, descended from resistant F_3 progenies, have shown remarkable freedom from infection. They have manifested a resistance comparable to that of Black Mesdag. Occasionally, smutted plants have been found in a few progenies. It may be noted, however, that the plants infected with loose smut usually occur in the same progenies as those infected with the covered smut, and their presence may be accounted for on the basis of natural crossing in the field. Some natural crossing between the parental varieties has been detected during the past few seasons, and such natural crossing may also occur among the hybrids. The field hybrids are readily recognized when black-hulled individuals are found in a variety or population that should be light-hulled. The field crossing, however, might have occurred in which pollen from a light-hulled parent reached a black-hulled individual but, if this happened, it is not so easily detected. It is quite possible that an occasional cross-pollination in the second generation plant would show up by giving rise to infected plants in the fourth generation. The destruction of the infected plants makes it impossible to determine what actually happened, and the loss of the infected individuals is a serious difficulty in some phases of the studies on smut inheritance.

DISCUSSION AND SUMMARY

During the past few years, several investigators have studied the inheritance of smut resistance in various oat hybrids. Since these results have been reviewed recently (Reed, 1932b), they need not be further considered here.

The three sets of hybrids—Hull-less \times Black Mesdag, Silvermine \times Black Mesdag, and Early Champion \times Black Mesdag—have shown a similar behavior in their reaction to the loose and covered smuts. The results with the F_2 generation of all three indicate that resistance is dominant and that segregation occurs on the basis of a three-to-one ratio.

The results with the third generation progenies harmonize quite well with the data secured for the second generation. It must be emphasized that there were three types of F_3 progenies, depending upon whether the F_2 plant had been inoculated with either loose smut or covered smut, or was uninoculated. In the first two groups, the F_3 progenies consisted approximately of one resistant to two segregating; with the F_3 progenies in the third group, there were three classes, approximating one resistant, two segregating, and one susceptible. There were found a few susceptible progenies in the first two groups where they might not be expected, but these may have been due to the failure to secure the infection of susceptible F_2 plants.

Nearly all of the F_4 progenies were descended from resistant F_3 families, and practically all of them were pure resistant. Occasionally smutted plants were found, but they may have been due to chance natural crossing in an ear-

lier generation. In all the hybrids, it is possible to find, in the F_4 generation, many progenies as resistant as the original resistant parent, Black Mesdag.

There was a marked parallelism in the inheritance of resistance to both smuts in the various F_3 progenies. By far the larger number showed a similar behavior to both loose and covered smut. These results suggest that the same factor, or closely linked factors, are responsible for the resistance and susceptibility in these hybrids.

It may be noted, however, that a higher percentage of infected F_2 plants was obtained in the loose smut series. The third generation progenies also gave, on the average, a greater percentage of infection with the loose, as compared with the covered smut. There were several cases where the reverse occurred, but in general it holds true that the higher percentages of infection were secured with the loose smut.

Results obtained with some other oat crosses may be noted briefly. Data on a hybrid between Gothland and Victor have been published (Reed, 1932a). Victor is susceptible to both smuts, while Gothland is susceptible to loose smut and resistant to the covered. Results with both the second and third generation plants show practically complete susceptibility to the loose smut. On the other hand, the results with the covered smut indicate that resistance is dominant and inherited on the basis of a three-to-one ratio.

In another paper (Reed, 1931) the results of experiments on the inheritance of smut resistance in hybrids of Gothland and Monarch are reported. These two varieties differ in their reaction to the two smuts; Gothland is very susceptible to loose smut and resistant to the covered, while Monarch is resistant to the loose smut and susceptible to the covered. Data were obtained on the second, third, and fourth generations, different sets of plants being inoculated with the two smuts. The data indicated that the factors for resistance to loose and covered smut are independently inherited in these hybrids. A third generation progeny resistant, segregating, or susceptible to the loose smut might belong in any one of these classes in its behavior to the covered smut. It was possible, however, to combine in the same selection or strain complete resistance to both smuts.

These results perhaps are not surprising in view of the fact that we are dealing with two independent parasitic organisms, and also with two oat varieties which show a marked difference in their behavior towards them. The results, however, are decidedly different from those obtained in the present crosses, in which resistance and susceptibility to the two smuts run parallel.

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SPECIES RELATIONSHIPS IN *EUPHORBIA* AS SHOWN BY THE ELECTROPHORESIS OF LATEX

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In recent years a number of attempts have been made in several fields of biology to place the taxonomic relationships of species upon a firm physico-chemical foundation. As an instance, the work of Mez and his school may be cited (Mez, 1922; Hoeffgen, 1922). These investigations have produced the so-called "Sero-diagnostischer Stammbaum" which arranges the families of plants on the basis of their immunological reactions (Mez and Ziegenspeck, 1926). Comparison of this "tree" with the taxonomic relationships already established shows that they are in most cases similar or identical. Likewise, it indicates that closely related plants have proteins which differ but slightly in their composition, and as morphological relationships become less, protein divergence increases. This work has drawn much criticism from Gilg and Schürhoff and their group, but later work by Boom (1930) and by Moritz (1928, 1929), who gives a critical review of the literature, has shown that, in the main, Mez' conclusions are sound. By using anaphylactic methods, Moritz (1932) and Moritz and vom Berg (1931) have even been able to analyze the components of the compound antigens used by Mez.

The investigations of Avery, Goebel, and Babers (1932) and those of Landsteiner and van der Scheer (1929) have shown that many complex proteins can be formed which differ only in the spatial arrangement of the groups about a single carbon atom. These proteins yield specific antibodies. This indicates that there may be many natural proteins which shade off into one another by slight gradations, yet each one would be characterized by immunological specificity. Wells (1929) has given a more detailed discussion of specificity.

In addition to the serological method of investigating protein specificity, there are physical chemical methods such as a comparison of isoelectric points. Protein-coated colloidal particles possess an electric charge, the electrokinetic potential (Freundlich, 1922). This can be measured by recording their mobility in an electric field, a method designated as electrophoresis. By varying the pH, the isoelectric point (point of no motion) can be reached. As this point has a definite value for each protein, it may serve as a means for relating and distinguishing proteins or other substances of an amphoteric nature. Also, since the numerical value of the isoelectric point is a function of the ratio

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between the strengths of the acidic and basic groups (Levene and Simms, 1923; Michaelis, 1926), its position may indicate their relative number. Two amphoteric substances may have the same isoelectric point on the pH scale, yet be totally different in composition. Therefore, such a method of identification could be used only to show similarities or differences between ampholytes within a natural group. This technique is a type of "referee" method, and thus it cannot be used to show relationships between unclassified forms, except possibly in a very tentative way.

Michaelis (1911), Beniasch (1912), de Kruif (1922), and others have found that the acid agglutination optimum (which corresponds to the isoelectric point) has a specific value which makes it possible to separate groups. Bacteria have also been separated by a comparison of their charges by Falk and his school (Falk, 1928). They have found that virulent strains of bacteria may have a higher electrokinetic potential than their non-virulent relatives. Of the four types of pneumococci causing lobar pneumonia, both the potential difference (P.D.) and the virulence for white mice and man were in the order $3 > 1 > 2 > 4$. Chapman (1929) has been able to separate different strains of *Bacillus coli* by comparing their potentials at a constant pH value, while Reed and Gardiner (1932) have compared complete mobility curves for both R and S types of *Mycobacterium leprae*. The differentiation of *Haemophilus pertussis* strains has been done by Shibley (1932) using these methods. Erythrocytes have also been shown by Abramson (1929) to possess a specific P.D. dependent on species. Not only the isoelectric points but also the shapes of the velocity curves plotted against pH can be used to study an amphoteric substance. The difficulty of distinguishing between two proteins of the same isoelectric point but different characteristics is obviated when the curves show differences in shape. The curves give a complete picture of the changes which ensue with change of pH. It has been shown by many workers, following Loeb (1922) and Abramson (1928), that inert colloidal particles in protein solutions assume the isoelectric point and electrokinetic properties of the protein itself. The nature of the particle is immaterial; only the adsorbed surface influences the reaction. The use of electrophoresis as a tool in this work has been summarized from a practical standpoint by Seifriz (1928) and by Prausnitz and Reitstötter (1931). For the theory, the reader is referred to Pauli and Valkó (1929) and to Smoluchowski (1921). The work in relation to bacteria is discussed by Mudd, Nugent, and Bullock (1932).

Particularly suitable subjects for investigation in this connection are latex particles. These colloidal particles occur naturally in living cells of many plants (Molisch, 1901; Bobilioff, 1919; Frey-Wyssling, 1932). The structure of *Hevea* latex particles has been studied by Freundlich and Hauser (1925) and by Hauser (1930) by means of microdissection. The particles were found to be pear-shaped with a liquid center surrounded by a shell of denser rubber. Hauser pictures a partial coating of adsorbed protein. Weber

(1903) seems to have been one of the first to suggest a protein-coated particle, but most later evidence indicates that the protein coating, although present, is incomplete. Freundlich and Hauser (1925) and Beumee-Nieuwland (1929) have offered most convincing proof that this is the case. The work has been summarized by Whitby (1920), Hauser (1930), Fisher (1930), van Harpen (1931), and Morris and Greenup (1932). Concerning the structure of latex particles in other genera, little is known beyond their microscopic pictures and, in some cases, chemical analysis (Hauser, 1930).

The present investigation was undertaken to see if resemblances between closely related species would be shown by the electrophoretic mobility curves of their latex particles.

The electrophoresis of latex was first investigated by Henri (1906), who found it moved to the anode and hence its particles possessed a negative charge. This has been utilized by industries to plate substances with latex (Sheppard, 1927; Prausnitz and Reitsötter, 1931). Belgrave (1923) appears to have been the first one to report a positive charge on latex after addition of acid. Belgrave (1923), Sheppard and Eberlin (1925), Rowland (cited by Dinsmore, 1926), and Twiss (1931) found varied isoelectric points for ammonia latex, depending on its degree of preservation. All of the values lie within the range of pH 3.0–pH 5.0 which includes the isoelectric points of most proteins (Mudd, 1925a; Pfeiffer, 1929). Fresh latex from *Hevea* has a more definite isoelectric point at pH 4.8 as determined by its acid coagulation optimum (van Harpen, 1931).

Unfortunately, fresh *Hevea* latex can be obtained only in the tropics; consequently, for this problem latex from various species of *Euphorbia* was studied. The genus *Euphorbia* is a large one and includes many species, all of which contain latex. Hence, it is particularly suited for work of this character. As far as known, there appears to be no literature on the electrophoresis of latex from this genus.

MATERIALS AND METHODS

Some of the plants in this work were grown from seed obtained from the Muséum National d'Histoire Naturelle in Paris and from the University of Warsaw, some were from the botanical gardens of the University of Pennsylvania, and others were collected locally. The latex was obtained by severing a leaf or nicking the stem with a clean razor blade and suspending the exuding drops in M/50 acetic acid-sodium acetate buffer mixtures in a dilution of approximately one drop of latex to 25 cc. of buffer. Both Mudd (1925b) and Abramson (1932) have shown that acetate buffers are preferable for this type of work, and since this buffer system has been used by other investigators in electrophoresis, all curves are confined to its pH range. Pauli (1920) has shown that strong acids yield anomalous results and cannot give a true electrophoretic isoelectric point, hence they have been avoided. Every buffer dilution was made immediately before each test and its pH measured

(with the latex in it) by the quinhydrone method (Michaelis, 1926; van Harpen, 1929, 1931). A "type-K" potentiometer and a saturated calomel cell were used in each case. The standards proposed by Clark (1928) were used to convert voltage to pH.

Measurements of electrophoretic velocity were made by the microscopic method with a modified Northrup-Kunitz (1925) apparatus after the design used by Mudd, Lucké, McCutcheon, and Strumia (1928). Three radio "B" batteries, giving approximately 135 volts, were connected at each end of the cell to non-polarizable electrodes of zinc in saturated ZnSO_4 . The electrophoresis cell was mounted with oil immersion contact over a Zeiss Wechselcondensor. A single cell was used throughout. A $28\times$ Zeiss ocular and a Bausch & Lomb 8 mm. objective combined working distance with sufficient magnification. The apparatus is shown in figure 1.

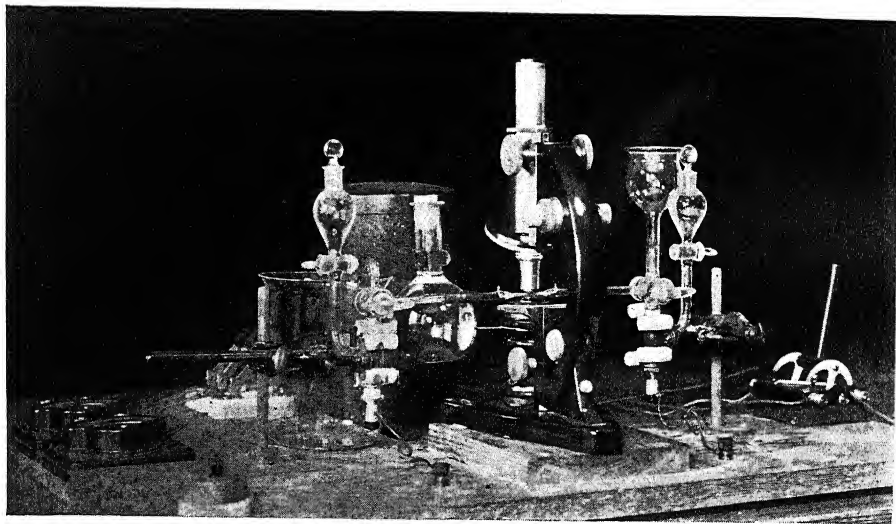


Fig. 1. The Northrup-Kunitz electrophoresis apparatus.

Since velocities are given in $\mu/\text{sec.}/\text{volt}/\text{cm.}$, the potential drop per cm. must be found. Following Ohm's Law,

$$\frac{L}{Q} = K = \frac{E}{RI}$$

where E is the potential across the chamber itself; L , the length of the chamber; Q , its cross section area; R , the specific resistivity of the fluid filling it; I , the current; and K , a constant dependent upon shape. For calibration,¹ the cell is filled with mercury and placed in series with a dry cell, a rheostat, and a Weston standard ammeter. A potentiometer is connected across the cell by small platinum electrodes led into it. The P.D. and current are read

¹ Thanks are due to Professor Charles Weyl for suggesting this method of calibration.

simultaneously and the resistivity of the mercury at room temperature is found from tables.

Having found K , it is used, in practice, in the equation

$$H = \frac{KRI}{L}$$

where H is the P.D. per cm. R is measured by a Wheatstone Bridge; I , by a milliammeter, which can be introduced into the circuit; and L is measured directly. The cell was flushed several times with part of the buffer before the latex suspension was introduced. Care was taken to prevent the inclusion of air bubbles. The current and resistivity were measured at the start. Velocity was measured with a stop-watch by determining the time required for a latex particle in sharp focus to travel between two lines of the ocular micrometer and back again; a Pohl mercury commutator was used to change the direction of migration when the particles reached the second line.

Due to the charge which the glass wall assumes against the water and the consequent electroendosmotic streaming when the circuit is closed, it is necessary to measure the particle velocity at definite depths to eliminate this source of error. Smoluchowski (1921) has formulated these levels for flat cells. These "stationary" levels lie at 0.21 and 0.79 of the total depth of the cell. At least five readings were made at each level, and the mean of these was taken to calculate the velocity in μ /sec./volt/cm.

Measurements were made at temperatures from 21° to 28° C. The temperature coefficient of velocity was found to be approximately 2 per cent per degree centigrade. This same coefficient has also been used by Abramson (1929, 1932) for protein-coated particles. To aid in comparison, all data were recalculated to a temperature of 25° C. Variations in the coefficient appear to lie outside its experimental error, so that the same value was used throughout.

Velocities were plotted against pH, all curves being drawn to the same scale. As shown by Abramson (1931), Freundlich and Abramson (1928), Henry (1931), and Sumner and Henry (1931), the Helmholtz-Lamb equation,

$$\zeta = \frac{4\pi\eta V}{HD}$$

(where H is P.D. per cm., D , dielectric constant, ζ , the electrokinetic potential, η , coefficient of viscosity, and V , the velocity—all units being electrostatic), is valid for insulating particles. Using this equation and making certain assumptions for η and D , the zeta potential can be calculated in millivolts by multiplying the observed velocity by 12.6 (Northrup and Cullen, 1922). It was thought better to express the results simply in terms of mobility.

Near the isoelectric point measurements of time are not very accurate, since the time needed to travel the required distance approaches infinity as

the velocity approaches zero. To avoid this, readings were made at small increments of pH, above and below the isoelectric point, until two suspensions were found having approximately equal and opposite velocities at very close pH values. These values were then reduced to terms of hydrogen-ion concentration, averaged, and re-converted to pH units. It was found that these values and the points at which the curves changed their sign (when interpolated) checked closely. In discussing results the abbreviation I.P. will be used for isoelectric point.

All the plants were tested by the ninhydrin and xanthoproteic reactions (Morrow, 1927) in the hope that these might afford a qualitative test for proteins. Other tests were not used because of possible disturbing factors in the latex. Of these two, the ninhydrin is thought to be of more value. Because of the small quantities of latex available, other, more accurate tests could not be applied.

RESULTS

Unfortunately, there is no recent general treatise on the species of *Euphorbia*. The monographs of Boissier (1866) and Berger (1907) are still the standard works in the field. Boissier systematizes the species by their seed and flower characters instead of their vegetative organs which, for convenience, are used by most other authors. Hence, Boissier's treatment probably represents a nearly natural grouping. His arrangement and key to the sub-groups will be followed closely in the discussion of the results of these electrophoretic experiments. For the section *Diacanthium*, Berger's classification will be used. Certain species represent the sole examples studied in their group due to a lack of available material. Other groups are better represented, and from these comparative data could be secured. It might be mentioned that all plants investigated gave a blue color with guaiacum, indicating the presence of oxidases.

Latex particles of *Euphorbia* are very small, usually less than 0.5μ in diameter. There is a variation in size between particles from the same plant as well as in the particles from different plants. In the electric field, however, all particles of the same latex move with the same velocity under constant conditions, independent of size or shape within, of course, the limits of experimental error. Abramson (1931) has shown this to be true with many other types of particles. Under similar conditions, the velocity of latex particles from different individuals of the same species showed little fluctuation, but it was not so constant as the I.P. or the shape of the mobility curve. The I.P. given by any specimen could be obtained from another individual of the same species to within at least ± 0.1 pH, and usually with greater accuracy. Neither source of seed nor environmental conditions of growth (soil, age of plant, etc.) appeared to have any effect on the results as long as the plants were in good, healthy condition. If, however, plants were in any way pathological or if other possible variations (levels in the cell, composition of solutions, temperature, etc.) were not carefully eliminated, results varied widely.

It was found that there is a considerable degree of correlation between the positions of the isoelectric points of related species. All species investigated possessed an I.P. in the range of the buffer used (pH 3.2–pH 5.9). However, certain species did not reverse their charges until the lower limit had been almost reached, so that their behavior on the acid side of the I.P. could not be determined. Curves of this type were extrapolated for a short distance (in dash lines) to indicate reversal.

Section X.² *Trichrostigma*

The curve for *E. fulgens* Karwinsk. is of the sigmoid type (fig. 2) with a symmetry unique among the plants studied. Its I.P. at pH 4.3 and its

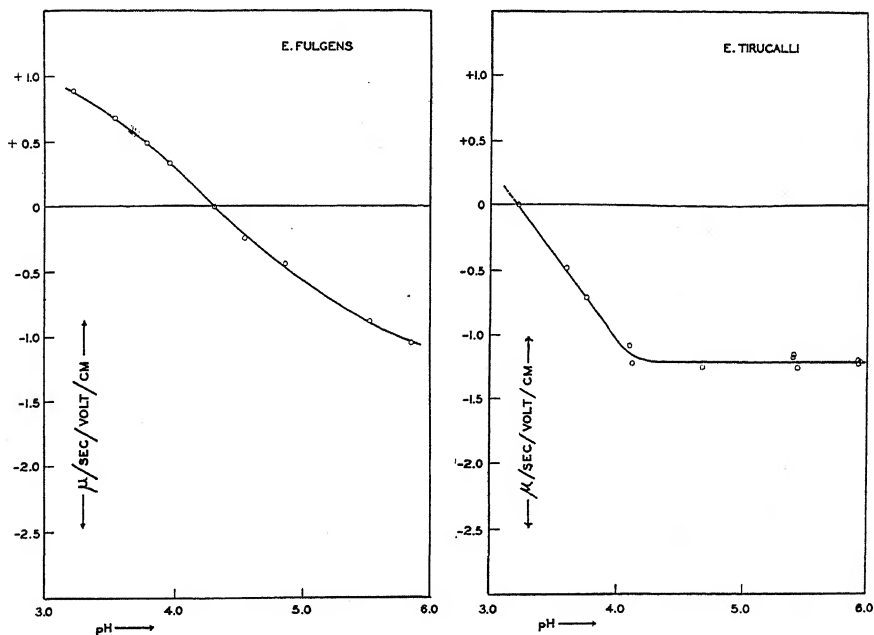


Fig. 2, 3. Fig. 2 (left). Mobility curves of latex particles from the section *Trichrostigma*. Fig. 3 (right). From the section *Tirucalli*.

shape set it apart from the other species just as it is set apart by taxonomy. Its protein content is relatively high, as shown by the ninhydrin reaction.

Section XV. *Poinsettia*

Three species of *Poinsettia* were investigated. The first species, *E. pulcherrima* Willd., was represented by three varieties: (1) the type, with ovate leaf and entire margin, and by two varietal forms; (2) red oak, named from its leaf shape; and (3) *alba*, named from its white bracts and petioles. All these forms are shown in figure 4 to be closely allied by the shape of their

² Numbers and symbols represent the classification of Boissier.

curves. The I.P.'s lie at pH 3.8 for *E. pulcherrima* and pH 3.9 for red oak and the white form. The curve of another species, *E. dentata* Mich., is next to that of *pulcherrima*. This is shown more clearly in figure 5, where the curves have been moved apart so that details may be observed. The I.P. of

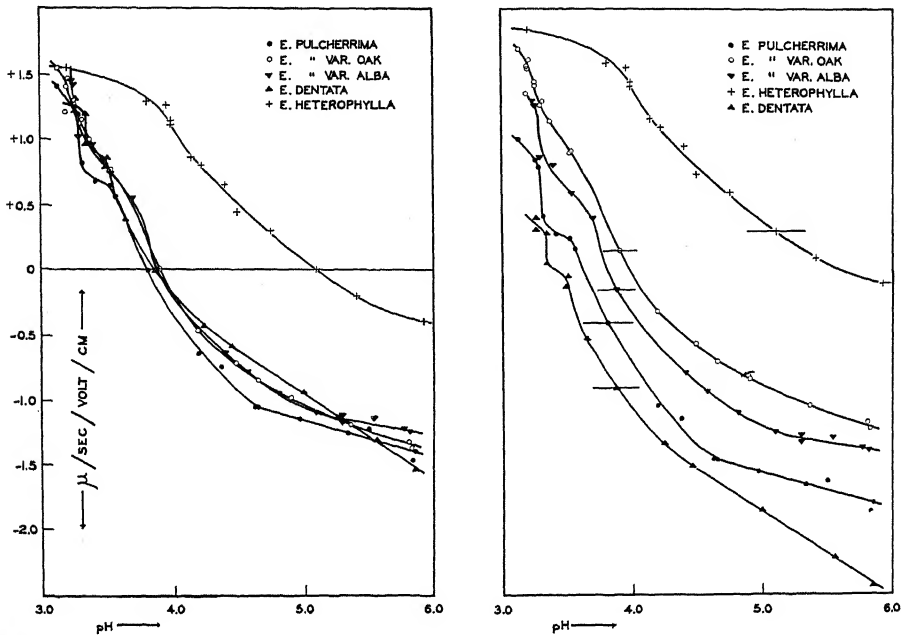


Fig. 4, 5. Fig. 4 (left). Mobility curves of latex particles from the section *Poinsettia*; all species plotted to the same scale. Fig. 5 (right). Curves spread out to show shapes.

E. dentata lies at pH 3.9, close to *alba*. Although the curve of *E. heterophylla* L. has somewhat the shape of the others, its I.P. is at pH 5.1, showing that its latex differs greatly from that of its supposedly closest relatives. It will be noted that as the I.P. increases the curves become smoother. Protein reactions are high throughout this group.

Section XIX. *Diacanthium*

§ 1. *Biaculeatae*

a. Splendentes.—*E. splendens* Bojer is in some particulars similar to the other members, at least on the basic side of the I.P., although it is not in the same taxonomic group (fig. 6). Its protein reactions are high and its I.P. lies at pH 3.85.

e. Trigonae.—*E. lactea* Haw. has an I.P. (pH 3.8) close to that of *E. splendens* and a high protein reaction, but the characteristics of its curve are not very close to those of the other members of this group, *E. grandicornis*

Goebel and *E. grandidens* Haw. Its curve on the basic side, however, does show some similarity to the others. *E. grandicornis* and *E. grandidens* are closely related taxonomically and have similar curves both in shape and in

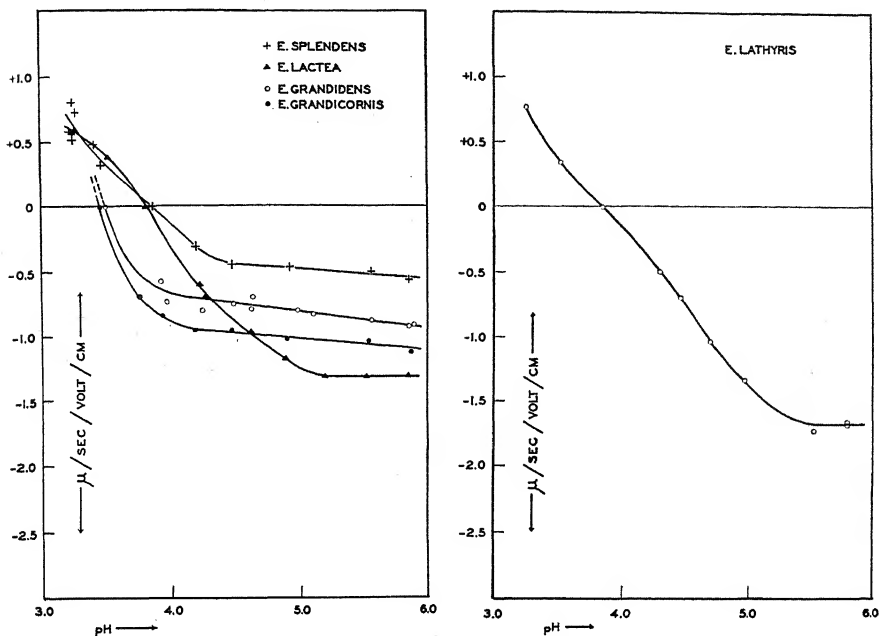


Fig. 6, 7. Fig. 6 (left). Mobility curves of latex particles from the section Diacanthium. Fig. 7 (right). From the section Tithymalus, sub-section Decussatae.

I.P.'s (pH 3.45 and pH 3.5 respectively). *E. grandicornis* gives a medium protein reaction while the reaction of *E. grandidens* is low.

Section XXII. *Tirucalli*

E. tirucalli L., the sole member investigated in this group, gave a curve peculiar to itself. It has a relatively low I.P. at pH 3.2 (fig. 3). Its protein content was low.

Section XXVI. *Tithymalus*

§ 1. Decussatae

E. lathyris L. gives a strong protein reaction and also strong color reactions with iron. Molisch (1901) and Douin (1930) mention the presence of tannins in this form. In this respect it was unique among all the plants investigated. Its I.P. lies at pH 3.85. See figure 7.

9. Galarrhaei

* Seeds smooth.

† Capsule smooth or obscurely and minutely tuberculate, not verrucose.

E. pilosa L.³ represents a perennial species, while *E. lagascae* Spreng. is an annual. The two are close in respect to I.P., which lies at pH 4.0 for *E. pi-*

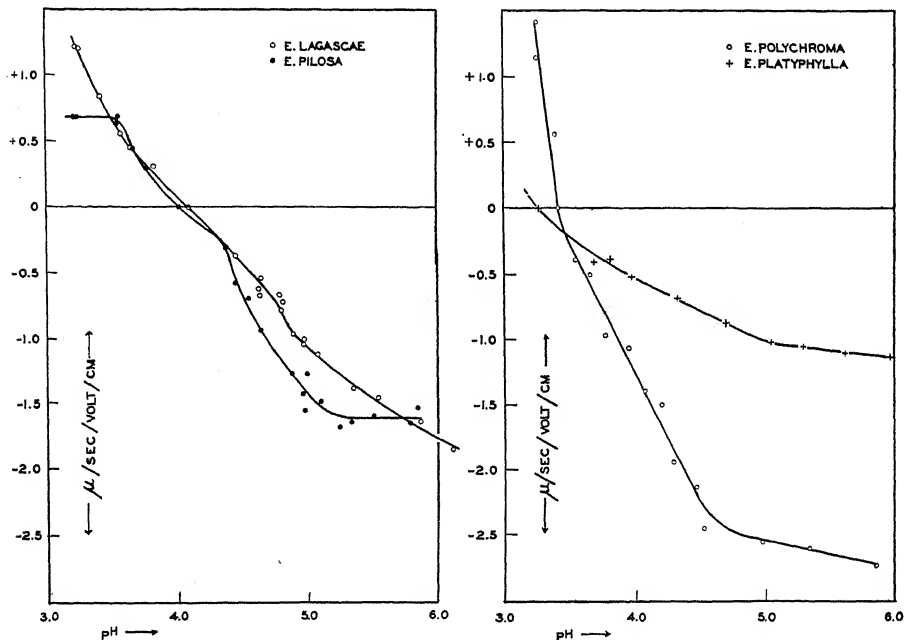


Fig. 8, 9. Fig. 8 (left). Mobility curves of latex particles from the section *Tithymalus*, sub-section *Galarrhaei*, with smooth capsules. Fig. 9 (right). From the group with warty capsules.

losa and pH 4.1 for *E. lagascae*. On the basic side of the range both curves show a hump, but *E. pilosa* reaches a plateau on both sides before *E. lagascae* (fig. 8). Protein reactions were low in this group.

††† Capsule covered by hemispherical, cylindrical or filamentous, elongated warts.

Here, *E. polychroma* Kern is perennial while *E. platyphyllus* L. is annual. The curves are of the same shape on the basic side (the acid side of the curve for *E. platyphyllus* could not be obtained, since it lies below pH 3.2). The magnitudes of the velocities of the two species are very different, however, for the latex particles of *E. platyphyllus* move very slowly, while those of *E. polychroma* flow faster than any other latex investigated (fig. 9). The I.P.'s

³ The name *E. pilosa* L. was taken from the seed envelope; unfortunately it could not be substantiated because the plants did not flower. Some uncertainty exists as to its specific identity, although from all characters available it seems undoubtedly a member of this group.

lie at pH 3.3 and pH 3.4 respectively. The ninhydrin tests gave a fairly strong protein reaction in the latex of both species.

10. Esulae

**** Seeds irregularly pitted, marked or reticulate-rugose.

E. segetalis L. and *E. pinca* L. are closely related taxonomically (*E. pinca* is a perennial, while *E. segetalis* is an annual). Their latex also behaves alike

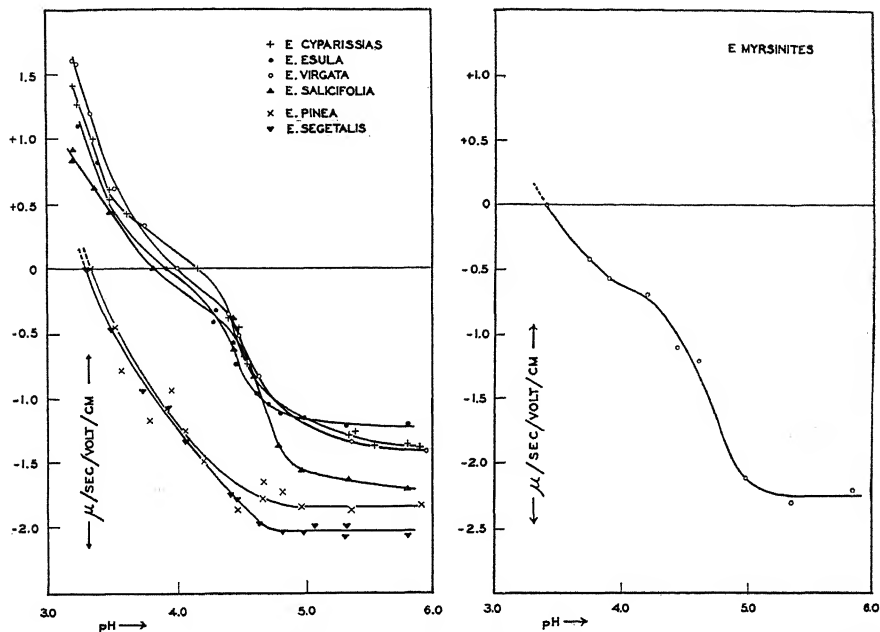


Fig. 10, 11. Fig. 10 (left). Mobility curves of latex particles from the section Tithymalus, sub-section Esulae. Fig. 11 (right). From the sub-section Myrsiniteae.

(fig. 10), and their I.P.'s both lie at pH 3.3. Both species give a moderate protein reaction.

**** Seeds smooth.

† Floral leaves free.

In this group are *E. virgata* W. & Kit. (I.P. at pH 4.0), *E. cyparissias* L. (pH 4.2), *E. esula* L. (pH 3.9), and *E. salicifolia* Host (pH 3.8). All these species have similar curve shapes with the same bends occurring in all four. *E. salicifolia* is slightly divergent, however. Protein reactions are low to medium in all four. Douin (1930) has shown by analysis that *E. cyparissias* has low protein content.

11. Myrsiniteae

* Seeds vermiculate-rugose.

E. myrsinites L. (fig. 11) is not very closely related to the others, and although at first glance its curve might seem to be similar to the preceding

group, the position of the I.P. at pH 3.4 does not indicate similarity. It gives a medium protein reaction. A table of the reactions is appended here for reference.

TABLE I. *Reactions of latex from different species*

Species	I.P.	Ninhydrin test	Xanthoproteic HNO ₃	NH ₄ OH	Number of plants studied	Velocity at pH 5.9 μ /sec/v/cm
	pH					
Tricherostigma						
<i>E. fulgens</i>	4.3	++++	+++	++++	2	— 1.06
Poinsettia						
<i>E. pulcherrima</i>	3.8	++++	++++	++++	3	— 1.41
<i>E. pulcherrima</i> var. oak	3.9	++++	+++	++++	2	— 1.35
<i>E. pulcherrima</i> var. alba	3.9	++++	+++	++++	2	— 1.25
<i>E. dentata</i>	3.9	++++	+++	+++	3	— 1.55
<i>E. heterophylla</i>	5.1	++++	+++	++++	5	— 0.39
Diacanthium						
<i>Splendentes</i>						
<i>E. splendens</i>	3.85	++++	+++	+++	2	— 0.54
<i>Trigonae</i>						
<i>E. lactea</i>	3.8	++++	++++	++++	1	— 1.30
<i>E. grandidens</i>	3.5	+	o	o	2	— 0.91
<i>E. grandicornis</i>	3.45	++	++	++	1	— 1.10
Tirucalli						
<i>E. tirucalli</i>	3.2	+	+	+++	2	— 1.21
Tithymalus						
<i>Decussatae</i>						
<i>E. lathyris</i> *	3.85	++++	++++	++++	3	— 1.69
<i>Galarrhaei</i>						
Capsule smooth						
<i>E. pilosa</i>	4.0	++	++	++++	2	— 1.62
<i>E. lagascae</i> *	4.1	+	++	+++	2	— 1.69
Capsule warty						
<i>E. polychroma</i>	3.4	+++	+++	++++	1	— 2.73
<i>E. platyphyllos</i>	3.3	+++	+	++	2	— 1.13
<i>Esulae</i>						
Seeds pitted						
<i>E. segetalis</i> *	3.3	+	+	++	3	— 1.85
<i>E. pinea</i>	3.3	+++	++	++	1	— 2.03
Seeds smooth						
<i>E. virgata</i> *	4.0	+++	++	+++	3	— 1.41
<i>E. cyparissias</i>	4.2	ochre	+++	++++	6	— 1.38
<i>E. esula</i>	3.9	+++	++	+++	4	— 1.21
<i>E. salicifolia</i>	3.8	++	+++	++++	1	— 1.73
<i>Myrsiniteae</i>						
<i>E. myrsinites</i>	3.4	+++	+	+	2	— 2.25

* Identified by the Royal Botanic Gardens, Kew.

o No reaction.

+ Very weak.

++ Pale color.

+++ Good color.

++++ Deep color.

DISCUSSION

The preceding results show that closely related plants have latex particles whose electrophoretic behavior and isoelectric points are similar or identical, while plants which are not members of the same taxonomic group have latices which differ in respect to these physico-chemical properties. As differences in electrophoretic behavior depend upon changes in the adsorbed surface of the particle concerned and not upon the size, shape, or composition of the particle itself, this indicates that the specific peculiarities of plants may extend even to the surfaces of their latex globules. This recalls the investigations of Reichert (1919), who found that plants could be grouped by the structure and chemical activity of their starch grains. More recently Bobiloff (1931) has formulated a key to *Hevea* clones by means of the color reactions of their latices. Calcium and magnesium salts are used to catalyze the activity of the natural oxidases present in *Hevea* latex. The enzymes then bring on a resultant discoloration differing for each clone. Attempts to repeat these experiments with *Euphorbia* species were unsuccessful. They indicate, however, the presence of other properties of latex which are specific in nature, in *Hevea* at least.

In the earlier use of electrophoresis as a taxonomic tool, the prevalent practice has been to group species of bacteria by a comparison of their mobilities in either an unbuffered medium, such as a physiological salt solution or distilled water, or in buffers at a pH near neutrality. Such methods would give inadequate results in such an investigation as is here described, for a comparison of the velocities at constant pH (as may be seen from the table) does not yield conclusive results concerning relationships of the latices. The isoelectric point affords a far better and more constant criterion, especially when accompanied by a complete curve of electrophoretic mobility with variation in pH.

An examination of the curves shows that even within the smallest taxonomic groups, differences sometimes occur in the behavior of the latex particles. A similarity is often shown between geographical distribution and these differences in electric charge. Considering first the poinsettias, it is shown in figure 5 that *E. dentata* has a curve which most closely follows *E. pulcherrima* and that the two varietal forms, red oak and *alba*, are more divergent. This indicates that *E. dentata* is more closely related to the type species, *E. pulcherrima*, than to the latter's varieties, which was to have been expected. The geographical range of *E. pulcherrima* is throughout Mexico and Central America. *E. dentata* is spread through the eastern United States, west to Ohio and Missouri, and south to Mexico. A third species, *E. heterophylla*, extends, however, from Illinois to Peru and Brazil (Boissier, 1866). Its curves and I.P. show that in this respect, at least, it is not very closely related to the other two species in the poinsettia group which have been investigated.

In the section *Diacanthium*, the species investigated fall into two groups—the *Splendentes* (*E. splendens*, a native of Madagascar [Berger, 1907]) and the *Trigonae*, three species of which are shown in figure 6. Of these three, *E. lactea*, which is different both in shape of curve and in I.P., is a native of the East Indies (Berger, 1907), while both *E. grandidens* and *E. grandicornis*, with similar curves and I.P.'s, come from South Africa (Brown, 1925).

Although *E. pilosa* and *lagascae* have nearly identical isoelectric points and curves which are to some extent alike, there are certain marked differences (fig. 8) which would not be expected in view of their close taxonomic relationship. However, their geographical distribution differs markedly. *E. pilosa* spreads through North Spain, South France, South Germany, Silesia, Austria, Hungary, Italy, Rumania, Middle and South Russia to Siberia, while *E. lagascae* is restricted to the Mediterranean region, Central and South Spain, Sardinia, and the Canary Islands (Hegi, Beger, and Zimmermann, 1930).

In the second group of the *Galarrhaei* (fig. 9) *E. polychroma* and *E. platyphyllos* differ widely in the position of their mobility curves but not in their curve shapes or I.P.'s. It is interesting to note that these two taxonomically closely related forms have different distributions. *E. polychroma* extends through east and south-east Europe from South Poland through the Balkans, while *E. platyphyllos* is spread throughout South and Middle Europe from Great Britain and North Spain eastward to Middle and South Russia, the eastern Balkans, Asia Minor, and North Africa (Hegi, Beger, and Zimmermann, 1930).

The two members investigated from the rough-seeded group of the *Esulae* (*E. segetalis* and *E. pinea*) show curves and I.P.'s (fig. 10) which are very much alike. Their geographical distributions are almost identical, ranging through the Mediterranean region and North Africa (Douin, 1930; Pax and Hoffmann, 1931). When this investigation was started, *E. segetalis* was called *E. amygdaloides* from the name on the seed envelope. *E. amygdaloides* belongs to a different group of the *Esulae* from *E. pinea*, and it seemed strange that the mobility curves and I.P.'s, when they were determined, should show a close relationship and not indicate this supposed difference. Specimens of "*E. amygdaloides*" were identified by Kew Botanic Gardens as *E. segetalis* and the difficulty was removed.

Of the smooth-seeded *Esulae*, *E. cyparissias* extends throughout Middle and South Europe and eastward to Siberia. Its northern limit is Cumberland (England), Denmark, South and Middle Sweden, Lithuania, Latvia, Esthonia, and Middle Russia, while it is bounded on the south by Middle Spain, South Italy, Albania, Macedonia, and South Russia. *E. esula* ranges over virtually the same area. On the north it reaches Scotland, Denmark, South Sweden, Lake Ladoga in Finland, Novgorod, and the Onega Valley, and on the south, North Spain, Middle Italy, the North Balkans, Rumania, and Middle Russia. It also extends to western Asia. *E. virgata* is found from Czechoslovakia, Poland, Latvia, Lithuania, and Esthonia stretching

south-eastward through the North Balkans in the south and through Middle and South Russia on the north to Siberia, up to Dsungarei and western Asia. *E. salicifolia* extends from Bavaria eastward through the steppe region from Austria and Hungary, Galicia, and the North and Middle Balkans to Bulgaria and South Russia (Hegi, Beger, and Zimmermann, 1930). All these four species have similar curves and I.P.'s. Of the four, *E. salicifolia* diverges most widely from the rest in geographical distribution and in curve shape.

With few exceptions, species closely related and having similar geographical distribution are marked by similar latex behavior; between species having different distribution there is much less correlation. Furthermore, between members of different taxonomic groups there is little, if any, similarity in curve shapes or isoelectric points. These peculiarities can only be accounted for by differences in the surfaces of the latex particles. These surfaces all contain an ampholyte to some extent, for they all reverse their sign of charge as the pH is lowered. There are at least two groups of substances generally present in *Euphorbia* latex which are known to be amphoteric. These are the sterols and proteins.

Cohen (1908) and Klein and Pirschle (1923) have investigated many species of *Euphorbia* and report the presence of sterols in the latex of all forms tested. Of the plants they investigated *E. pulcherrima*, *E. cyparissias*, *E. lathyris*, *E. splendens*, and *E. myrsinites* were found to contain these compounds. Klein and Pirschle also claimed that euphorbon, a resin constituent found throughout the entire genus, is a sterol, but neither Bauer and Schenkel (1928) nor Müller (1929) could confirm this. Müller, however, believes that sterols are present. Many authors, following Belgrave (1923), have found sterols in *Hevea* latex and rubber (Whitby, Dolid, and Yorston, 1926; Bruson, Sebrell, and Vogt, 1927; Frey-Wyssling, 1929; van Harpen, 1931). There are but few references to the electrophoresis of sterols. Eagle (1930) finds the I.P. of cholesterol to lie between pH 2.1 and pH 3.6, while Remesow (1930) gives pH 3.2 as the value of the I.P. of the pure substance. Flocculation experiments by Rona and Deutsch (1926) on cholesterol show a maximum flocculation at pH 2.4–pH 3.2. Van Harpen (1931) finds the flocculation optimum of the natural sterols in the acetone extract of *Hevea* latex to be at pH 3.02. The I.P.'s of the proteins generally lie between pH 3 and pH 6 (Mudd, 1925; Pfeiffer, 1929; Tiselius, 1930), the range within which latex I.P.'s are found.

As far as our present knowledge is concerned, there are several possibilities as to the structure of the particle surface. The surface for each species may be composed of a single specific protein. Any differences in curve shape would then be due to specific differences in the chemical or physical arrangement of these proteins. Abramson (1932) has shown that adsorption upon inert surfaces does not change or tie up any radicals which function in the electrophoresis of the free protein. Hence, if specific differences are expressed by electrophoretic behavior, they must be due to a change in number

or arrangement of the *ionized* groups of the protein molecule. Tiselius (1930) points out that in solutions of a single homogeneous protein there is no pH value near the I.P. at which particles move half to one pole and half to the other but at any value they are either charged all positively, all negatively, or all discharged. The latex particles investigated did not do this. The I.P. could only be determined as that point at which approximately half of the particles in the field moved to one pole and half to the other at an extremely slow rate. This is an indication that the coating is incomplete or else not composed of one homogeneous protein.

In shape and in position of the I.P. (above pH 3.8), the curves for *E. fulgens*, *E. heterophylla*, *E. splendens*, *E. lactea*, and *E. lathyris* appear to come closest to the typical curves for particles coated with a single protein (Abramson, 1928, 1932; Stearn and Stearn, 1931; Tiselius, 1930). All these species gave a high reaction for protein.

On the other hand, instead of one protein, we may have mixtures of (1) several proteins or of (2) proteins with sterols or other compounds, not as yet known to be amphoteric, upon the surfaces of the particles. Investigations on the latex of *Hevea* show that the particle rarely, if ever, has a complete coating of protein (Whitby, 1920; Hauser, 1930; van Harpen, 1931). Stearn and Stearn (1931) and Vlès (1924) have considered the electrophoretic behavior of mixtures of two or more ampholytes and find that each mixture has an electrophoretic I.P. between the I.P.'s of its constituents. The individual I.P.'s are not shown on the curve, only their resultant. It is interesting to notice that *E. dentata* and *E. pulcherrima* with its varieties yield high protein reactions and curves which are strikingly similar to the theoretical curve for a mixture of two ampholytes with no mutual combination, as given by Stearn and Stearn. It is possible that these species thus show the presence of approximately equal amounts of both sterols, known to be present in abundance in *E. pulcherrima*, and proteins. If such a mixture be present, it is a constant one for each species.

If the species with I.P.'s below pH 3.5 are listed, it is noted that, with the exception of *E. myrsinites* and *E. polychroma* (both near the arbitrary border line with I.P.'s at pH 3.4), all the forms have a curve shape somewhat alike. This is seen in *E. grandidens*, *E. grandicornis*, *E. tirucalli*, *E. platyphyllos*, *E. segetalis*, and *E. pinea*. All these species yielded a medium or low test for proteins. Their I.P.'s are all at low values below the usual range for proteins and in that found for sterols. It is probable that in these cases sterols or unknown non-protein ampholytes are the chief coating of the particles. In the case of *E. polychroma* protein reactions are slightly higher, and possibly these cause an altered reaction.

The curves of *E. myrsinites*, the members of the smooth-seeded *Esulae*, and *E. lagascae* and *E. pilosa* are difficult to explain. Their protein reactions are extremely varied and their curve shapes are very irregular, a feature not

characteristic of pure protein. It is again possible that a mixture is here present.

In general the results here obtained can be explained best by assuming that the latex particles of *Euphorbia* vary greatly in their surface structure as we pass from species to species but that the composition of the particles obtained from any one species is constant and specific, and resembles, in constitution, those of its nearest taxonomic neighbors.

SUMMARY

1. The isoelectric points and electrophoretic mobility curves for the latex particles (suspended in buffers) of twenty-one species of the genus *Euphorbia* have been determined by means of a Northrup-Kunitz electrophoresis apparatus.

2. The species investigated are: *E. fulgens*, *E. pulcherrima*, *E. pulcherrima* var. red oak, *E. pulcherrima* var. alba, *E. dentata*, *E. heterophylla*, *E. splendens*, *E. lactea*, *E. grandicornis*, *E. grandidens*, *E. tirucalli*, *E. lathyris*, *E. pilosa*, *E. lagascae*, *E. polychroma*, *E. platyphyllos*, *E. segetalis*, *E. pinca*, *E. virgata*, *E. cyparissias*, *E. esula*, *E. salicifolia*, and *E. myrsinites*.

3. These values of isoelectric points and mobility curves appear to be constant and specific for each species and do not depend upon environmental factors so long as specimens are in good, healthy condition.

4. These curves, representing the variation of the electrokinetic potential (plotted as velocity) with change of pH, can be grouped into families whose members have similar positions and shapes.

5. These families of mobility curves of latex particles correspond to the taxonomic groups already established for the genus.

6. If the isoelectric points of the latex particles of the several species are grouped according to the natural taxonomic arrangement, closely related plants are found to have isoelectric points at close or identical pH values.

7. A marked correlation was found between similarities in geographical distribution and curve shape.

8. Relations between species are not clearly shown when electrophoretic mobility values are taken at constant pH, since this method affords no indication of the rest of the mobility curve or of the I.P.

9. All plants investigated showed the presence of oxidases by the guaiacum reaction.

10. All plants studied gave an isoelectric point in the normal range on the pH scale in which protein isoelectric points occur. This is an indication that the surface of the latex particle is at least partially coated with protein in most cases.

11. Latex particles of *Euphorbia* behave as though some species are almost completely coated with a single protein, some with several proteins or mixtures of proteins with other ampholytes, possibly sterols, while other species seem to have an almost completely non-protein surface.

12. In spite of these variations between species, each species acts as though its latex particle surface is constant in composition and little influenced by individual variations.

13. The method of electrophoresis is suggested as a useful tool in studying the taxonomic relationships and latex particle structure of laticiferous plants.

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LOPHIOSPHAERA (GLONIUM) VELATA, WITH A CRITICAL
STUDY OF ITS SEPTONEMA MULTIPLEX STAGE¹

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Despite recent investigations (Bisby, 1932; Lohman, 1933), a number of species of hysteriaceous fungi, described as new in the earlier literature on the fungi of temperate North America, have not been studied critically either in regard to the accuracy of diagnoses and the validity of names or in regard to the probable affinities as shown by their imperfect stages. One such species, *Glonium velatum*, was described by Ellis and Everhart (1892) on specimens collected (Langlois) in Louisiana. This species, since then, has been reported only by Underwood and Earle (1897) for Alabama. That it is more widely distributed than the two records would indicate is shown by the present report² which extends the range of the fungus to South Carolina, Florida, and Georgia.³

This report is concerned with the development of the fungus in culture and with the characteristics of its perithecial, pycnidial, and hyphomycetous stages. It presents certain necessary revisions in the description of the perithecial stage, the consideration of which has resulted in the inclusion of the fungus in the Lophiostomaceae rather than the Hysteriaceae. It records for the first time the features of the pycnidial stage and presents the evidence which reveals that the conidial development mentioned by Ellis and Everhart in their description and referred by them to *Dendryphium* is only another aspect of the fungus which they described and is the same as the Hyphomycete, *Septonema multiplex* B. & C. (Berkeley, 1874).

¹ Contribution No. 120 from the Laboratories of Cryptogamic Botany, Harvard University.

² Report on certain work done by the writer during the tenure of a National Research Council Fellowship, 1931-32, under the sponsorship of Professor William H. Weston, Jr., and presented at the Atlantic City meeting of the Mycological Society of America, December, 1932.

³ South Carolina: Occurring (as *Septonema multiplex* B. & C., with perithecia associated) within cavity of decaying trunk, *Quercus alba* L., Society Hill, October, 1849 (Curtis 2751), and on weathered wood, *Nyssa*, Society Hill, 1853 (Curtis 4033).

Florida: Occurring (with *Septonema multiplex* B. & C. associated) on decaying wood, *Quercus virginiana* Mill., Plymouth, March, 1893 (collection, in Farlow Herbarium, by W. C. Sturgis).

Georgia: Occurring (with *Septonema multiplex* B. & C. associated) on decorticated wood of *Quercus montana* Willd., Princeton, February, 1932 (collection by J. H. Miller). The writer gladly acknowledges the aid of Dr. Miller in obtaining specimens of this fungus suitable for its cultivation.

PERITHECIAL STAGE

A comparative study of collections, particularly the fragmentary, weathered specimens from Louisiana (Langlois 2220 in the Farlow Herbarium) and those collected in Georgia, has shown the original description to be inadequate and in need of revision. Its most serious inadequacies pertain to features paramount in the recognition of the general affinities of the species. These shortcomings, together with the more accurate interpretations of the material, may be briefly summarized. (1) The laterally compressed, subglobose, linearly ostiolate perithecia (fig. 1), deep-seated because of an en-

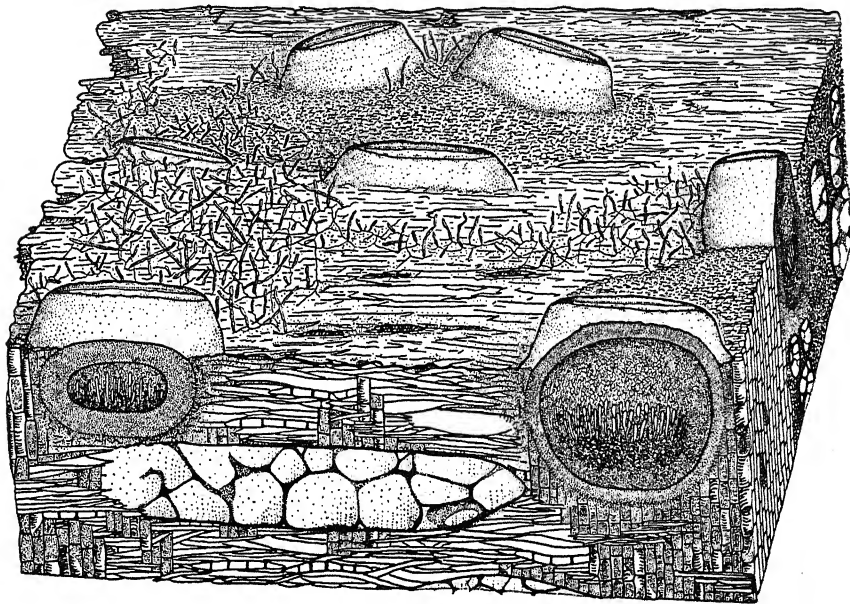


Fig. 1. Illustrating diagrammatically the habits of the perithecial and conidial stages of *Lophiosphaera velata* as they were observed on decorticated wood of *Quercus montana*; about $\times 65$.

doxylous origin and only occasionally appearing to be superficial even though the surrounding wood has been wasted by decay, do not attain one millimeter in length as stated by Ellis but measure (0.3) 0.4–0.6 mm. They measure (0.15) 0.2–0.3 mm. in width and (0.28) 0.35–0.58 mm. in height. (2) They may or may not be veiled by the dark web of the *Septonema*—not *Dendryphium*—stage; but when this stage is little developed, it is ordinarily only the full-length ostiolate summit of the perithecium which extends above the surface of the blackened wood. (3) The endoplasmic structure of the mature ascospore (fig. 2, A–D) with or without large guttulae is such that one may expect to encounter specimens the spores of which are more likely to be interpreted as phragmosporous rather than didymous, especially if protoplasmic

stains such as Cotton Blue or Light Green are employed in the microscopic examinations. This condition of the endoplast is manifested further by (a) the wall of the spore, since two slight constrictions are present, one in each half, and by (b) the development of definite cross septa at the secondary constrictions in the early stages of germination of the mature spore. The secondary constrictions are not noted in the original description, but on the basis of the presence of several large guttulae in some spores, a potential phragmosporous condition is mentioned questionably.

Several of the aforementioned features merit further discussion.

First, if a possible close relationship between this fungus and species of *Glonium*, to which genus Ellis referred it, be assumed, it must be admitted that the endoxylous origin of the perithecia and the extent to which they remain embedded at maturity (fig. 1) are habits not seen in species of *Glonium* but regularly observed in members of the Lophiostomaceae. Possibly the least superficiality of the hysterothecium known in *Glonium* is that exhibited by *G. lineare* (Fr.) De Not., a species with decidedly elongated fructifications, and by *G. parvulum* (Ger.) Cooke, a species with oblong to slightly elongated ones. Even in these species the hysterothecia are more or less superficial from the beginning, relative to the texture and firmness of the wood upon which

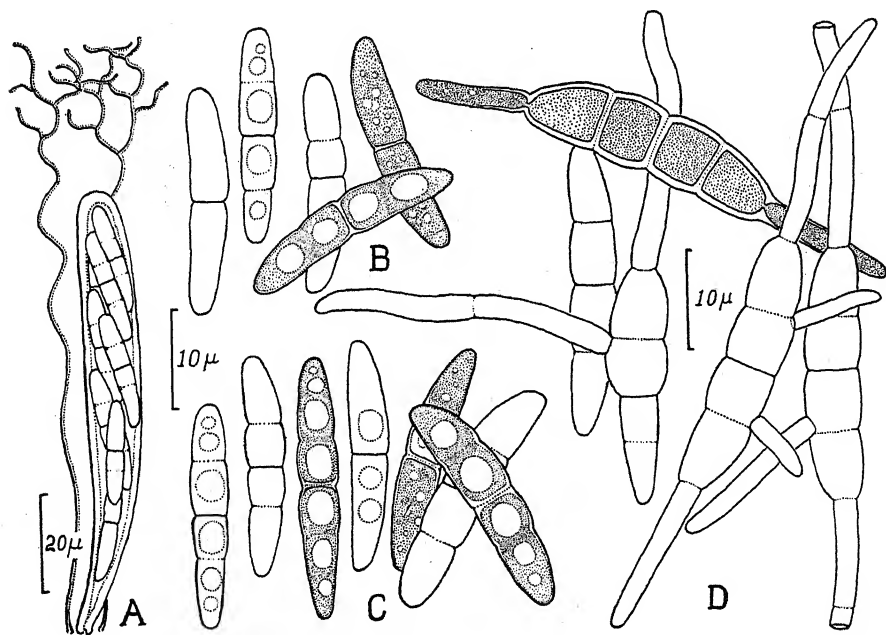


Fig. 2. Illustrating the features of the hymenial elements and the manner of germination of the ascospores of *Lophiosphaera velata*. A, C, and D represent the Georgia collection; B, the Louisiana collection, *Langlois 2220*. Shaded figures (B-D) illustrate median sections of stained spores. Approximate magnifications: A, $\times 650$; B-D, $\times 1300$.

they occur. It seems, then, that in referring this fungus to *Glonium*, Ellis perhaps was impressed unduly by the hysteriform ostiolate summit of its perithecium.

Second, although the ascospores of the fungus may appear to be 2-celled, they are potentially 4-celled as in *Lophiosphaera viticola* Sacc. and in species of *Lophiotrema*. When the mature ascospore is stained with Light Green in a lacto-phenol mounting medium, the endoplasm shows strong affinity for the stain, while the wall, the guttulae, and the cross septum remain clear (fig. 2, B, C). Then, at the primary constriction the spore shows a well-developed septum; at the secondary constrictions, only regions of dense protoplasmic content. The stained germinating spore (fig. 2, D), however, shows a definite septum at each secondary constriction. Since this change occurs during the swelling of the spore previous to the development of germ tubes, the grounds are ample for the statement that one may expect to encounter specimens the spores of which are more likely to be interpreted as 4-celled rather than 2-celled. Furthermore, it should be noted that germination in a small percentage of the spores under observation occurred without the development of the two secondary septa. Such spores, undoubtedly less mature, developed only polar germ tubes.

The close agreement between the collections from Georgia and Louisiana with respect to the measurements of asci and spores is worthy of note. In each the asci measure (75) $80-90 \times 8-10 \mu$. In the material from Georgia the ascospores measure (20) $22-25$ (27) $\times 3.5-4.5 \mu$, whereas an equal number (25) of records shows the spores of the Louisiana material to be (20) $21-23$ (25) $\times 3.5-4.5 \mu$, both sets of measurements approximating reasonably well the range given by Ellis—namely, $20-25 \times 3.5-4.5 \mu$.

DEVELOPMENT OF THE FUNGUS IN CULTURE

By application of the procedure described in a previous paper by the writer (1931, p. 144), utilizing the same medium, ascosporous isolations of the collection from Georgia were made to ascertain the relationship of the associated hyphomycetous stage. The fungus was then cultivated on a 2 per cent agar, maltose malt extract medium (*op. cit.*, p. 147) and on a 2 per cent agar, heavy (60 g. to the liter) oatmeal medium, in addition to that used in its isolation.

With respect to cultures held at $20-25^{\circ}\text{C}$. on each of the media mentioned, the mycelia developed very slowly, having reached an average radial growth of about 2.5 cm. at the close of the second month, and, with their marginal growth confined to the medium, produced mats which soon became black even though at first they were covered sparingly by olivaceous, cottony, aërial tufts. This change was effected by the development of the pycnidial and hyphomycetous stages. Ordinarily, upon the central surface mat of cultures in the light on the maltose medium, numerous pycnidia were developing by the

fourth week, but they were soon concealed by the profuse conidial development. As the cultures increased in diameter, usually several zones of maturing pycnidia bordered the enlarging area of conidial production.⁴

The pycnidia arise from individual primordia within the surface layer of the mycelial weft, and as they mature, they become closely crowded and quite superficial (pl. 1, fig. 5, 6). They present no unusual features in their morphological development.

The hyphomycetous conidia occasionally arise from the basal portions of pycnidia, or terminally on little-branched aërial hyphae, but for the most part they develop from short lateral outgrowths of the branched hyphae of the surface layer. A single chain may be composed of one or even of five or six conidia, the conidia and consequently the chains themselves varying greatly in the number of component cells and in length (see pl. 1, fig. 9). The chains develop by the elongation and single division of each successive terminal cell. Basal cells of a chain are thus the oldest; usually, they are larger and richer brown than those of the apical conidium. This condition is the converse of that described by the writer for the *Septonema spilomeum* stage of *Hysterium insidens* Schw. (1933a) and the *S. toruloideum* stage of *Mytilidium scolecosporum* Lohman (1933b), forms in which chains of initial conidial cells initiate conidial filaments and in which the apical conidium of the chain matures first. It is evident, then, that in any critical comparative studies on forms of *Septonema* the manner in which the conidial chain develops must be considered.

The filament of catenated cells in *Septonema multiplex* may be given several interpretations. (1) It may be interpreted as a chain of one or more conidia, each of which is the result of some stimulus for the division of an initial conidial cell that has originated from the supporting hypha in the case of the basal conidium and from the apical cell of the conidium formed last in the case of the remaining conidia in the chain. With this interpretation the conidium is delimited by prominent constrictions which serve as points of fragmentation in the chain. (2) The filament may be considered as a single, phragmosporous conidium similar to that of *Helicoceras* (Linder, 1931) except for the fact that at maturity it fragments at the septa where growth was temporarily arrested. (3) It may be considered as a chain of rather firmly bound, one-celled conidia which are disseminated in small groups. Without experimentation designed to control the development, attained size, and number of prominent constrictions in a filament, the observations made on the cultural habit and development of the fungus support the first of these

⁴Inasmuch as this fungus is known only from the southern states, its behavior in culture at temperatures lower than 20°C. is worthy of note. Examination at the close of the second month of transfers which had been made to the maltose medium and held (in the dark) at 10 (± 2)° and at 15 (± 2)°C. showed, at the lower temperature, no appreciable growth (although the inoculum remained viable) with neither pycnidia nor *Septonema* conidia present, and, at the higher temperature, a radial growth averaging half that in cultures at 20–25°C. without pycnidia but accompanied by scattered, short chains of conidia.

interpretations. It, fortunately, is the most practical of the three for dealing with the stage taxonomically.

PYCNIDIAL STAGE

As produced on culture media, the mature pycnidia are spherical to conic, ostiolate, and measure (60) 70–85 μ in diameter and (75) 85–105 μ in height (pl. 1, fig. 5, 6). They are black, subcarbonous, and their walls are complete, being composed of several rows of comparatively loosely interwoven branching hyphae, the outermost of which are broader, short-celled, thick-walled, and dark. The wall of the pycnidium is from 6 to 12 μ thick and lined within in both its lateral and basal portions with a palisade of minute, simple, clavate, closely clustered spore-bearing cells (pl. 1, fig. 7). The latter measure 6–8 μ in length, and 1.5–2 μ in diameter near their bases, taper apically, and bear singly at their tips hyaline, ovate to inequilateral or elliptic-oblong pycnidiospores which measure 1.5–2 \times 1–1.5 μ .

This particular combination of features does not conform to that of any of the pycnidial stages already described (Lohman, 1933a) for some 19 species of the Hysteriaceae. As compared with the pycnidial stages of the few species of *Glonium* for which that stage is known, it differs from that of *G. parvulum* (Ger.) Cooke (Lohman, 1931) and *G. stellatum* Muhl. ex Fr. (Lohman, 1933a) in the much smaller size, in the nature of the wall, which is filamentous rather than parenchymatous, and in the absence of a subulate beak; from that of *G. lineare* (Fr.) De Not. (Lohman, 1933a) in the smaller size of the pycnidium, spore-bearing cells and pycnidiospores, in the absence of elongate, sterile filaments among the spore-bearing cells, and in its superficial rather than locular development upon the same medium in the laboratory.

The pycnidial stage was not found on any of the field material examined. It probably occurs superficially on the less decayed wood, preceding the conidial and perfect stages of the species. In its systematic classification it is of the *Plenodomus* (Diedicke, 1911) and *Hysteropycnis* (Hiltzer, 1929) type.

SEPTONEMA STAGE

The hyphomycetous stage of this fungus apparently was collected first by Curtis in South Carolina (see footnote 3). It was listed by Curtis (1867) as *Septonema mutiplex* but was not described until 1874, at which time another collection from South Carolina (1563 by Ravenel—unseen by the present writer) was cited (Berkeley, 1874, p. 16). Since these early records the only additional reference to *S. mutiplex* is that by Cooke and Harkness (1881), who listed it as occurring on the bark of *Eucalyptus* in California, citing two collections. The conidial stage of *Lophiosphaera velata*, then, as a form species of the Hyphomycetes, has remained little known.

When Ellis and Everhart (1892, p. 685) encountered the stage in describing the perithecia of the species, they referred to it as follows: "The perithecia are imbedded in and covered, except [at] the apex, by a thin,

black, felt-like layer (*Dendryphium*) consisting of brown, branching hyphae, with abundant, cylindrical, multiseptate, brown, catenulate conidia $25-75 \times 5-6 \mu$, borne on short, upright branches. The septa in the conidia are about 4μ apart and there is a more or less distinct constriction at each septum." This diagnosis, despite the fact that it is incorrect in referring the stage to *Dendryphium*, is more exact than Berkeley's brief description (1874). Nevertheless, in the present study it proved to be exceedingly inadequate for comparing carefully the significant collections of this stage.

TABLE I. Summarized records of lengths and number of component cells of 250 conidia from each of three collections of "*Septonema multiplex*" and from ascosporeous cultures of *Lophiosphaera velata* (sources of materials are given in the text)

Classes (μ)	Number of conidia in 250				Classes (number component cells)	Number of conidia in 250			
	Collection		Cults.			Collection		Cults.	
	I	II	III	IV		I	II	III	IV
5-14	28	10	21	8	2-4	39	28	49	13
15-24	62	57	80	43	4-6	60	50	65	43
25-34	52	45	58	35	6-8	52	47	51	41
35-44	23	39	29	27	8-10	26	39	29	28
45-54	32	47	27	29	10-12	21	34	20	20
55-64	17	11	5	21	12-14	16	13	8	18
65-74	8	16	13	11	14-16	14	17	13	19
75-84	13	15	4	12	16-18	4	13	2	4
85-94	3	3	2	7	18-20	7	4	5	8
95-104	7	2	4	6	20-22	6	1	1	15
105-114	2	1	1	10	22-24	3	2	1	4
115-124	2	2	1	7	24-26	1	0	0	6
125-134	0	0	1	4	26-28	0	1	2	3
135-144	0	1	0	2	28-30	0	1	0	3
145-154	0	1	0	5	30-32	0	...	1	6
155-164	1	...	3	4	32-34	1	...	2	5
165-174	0	2	34-36	0	4
175-184	1	6	36-38	1	2
185-194	3	38-40	2
195-204	1	40-42	1
205-214	3	42-44	1
215-224	0	44-46	1
225-234	1	46-48	2
Totals	250	250	250	247*	Totals	250	250	250	249†

* The three additional conidia, so extreme in length that they are excluded from the above classification, measure 265, 275, and 345μ .

† The number of component cells in the conidium not included is 62.

The associated *Septonema* stages in the Louisiana and Georgia collections of *L. velata* were seen at once to agree reasonably well with the original collections of *S. multiplex* so far as general habit and such features as width, color, and orientation of conidia were concerned (pl. I, fig. 1-4). That each presented a condition of unusual variability with respect to length of conidia and the number of component cells was also apparent. In order to investigate the extent of the apparent variation and to have available for systematic

studies data more expressive of these diagnostic features than those presented in the descriptions by Berkeley (1874) and by Ellis and Everhart (1892), the lengths and the corresponding numbers of component cells were recorded for 250 conidia of each of the three important collections—namely, (I) the South Carolina collection, Curtis 2751 of 1849, (II) the Georgia collection of 1932, and (III) the Louisiana collection, Langlois 2220 of 1890. Besides, for further comparison, similar data (IV) were obtained for 250 conidia taken from mono-ascospore cultures of II. The records are classified in table 1 and presented in graphic form in figure 3. They are summarized in part in table 2.

An examination of the data shows that a wide range in conidial length, with lengths near the lower limit of the range predominating, is a constant feature of this *Septonema* stage and that a fairly uniform ratio of length of conidium to the number of its component cells obtains in each of the collections, despite the extreme variation in length. With respect to the field collections, it is to be noted that although the conidia range on the average from 10 to 160 μ in length, approximately half of them fall within the nar-

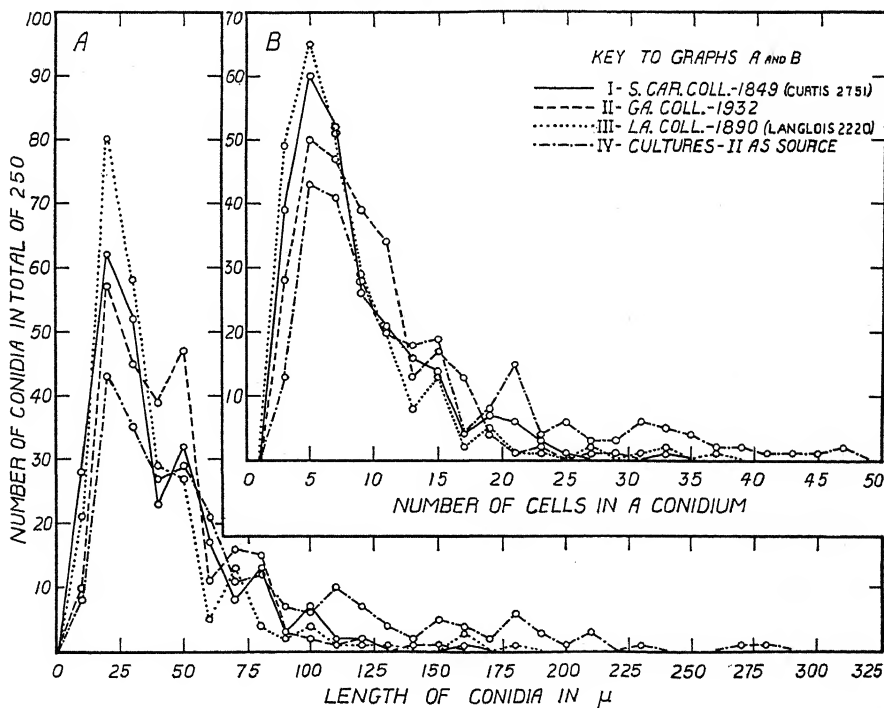


Fig. 3. Comparison of the records (summarized in table 1) on length and number of component cells of the conidia of four significant samples of *Lophiosphaera velata*; A, variation of conidia in length; B, variation of conidia in number of component cells. In plotting the records for IV a conidium measuring 345 μ in length and containing 62 cells has been disregarded in each graph.

lower range of 15 to 35 μ ; and furthermore, that although the conidia range from 2 to 32 in the number of cells contained, a range of 4 to 8 accounts for about half in each collection. The distribution of the conidia obtained in culture, however, is somewhat different. This the graphs show clearly. The departure of the curve for conidia obtained in culture from those for field collections is probably due to the fact that the uniform, or less fluctuating, conditions of the laboratory result in the production of a greater number of long conidia, and to the fact that conidia in cultures are not subject to such mechanical fragmentation as is brought about by weathering which, in the field material, results in a higher number of short conidia in the records even though care is taken to disregard as far as possible the measurement of conidial fragments.

TABLE 2. *Certain biometric constants of length of conidia in three collections of Lophiosphaera velata*

Collection	Mean	Median	Approximate mode	Standard deviation	Mean; ratio of length to number of cells
	μ	μ	μ	μ	
I	38.7	30.5	23	25.8	4.85
II	41.5	37.0	25	23.7	4.95
III	35.9	31.0	22	26.7	4.85

The significance of the data for the conidia from cultures is in that they clearly show, when compared with the data for the field collection from which isolations were made, the potentialities of the organism in regard to variation in the size of the conidia. With this knowledge of the variation possible in this conidial stage the biometric constants recorded in table 2 may be interpreted as indicative of a close similarity of the three collections being compared. Additional field collections would undoubtedly yield in each case biometric characteristics approximating these. The difference between the mean and the mode in the present collections may appear unusually large, but a large difference is to be expected in the type of variation presented by *S. multiplex*.

The conidial stages of the Louisiana and Georgia collections of *Lophiosphaera velata*, then, are in rather close agreement with the type material of *Septonema multiplex* B. & C. in regard to all of the important diagnostic features. In view of the biometrical characteristics presented herein, the type material of *S. multiplex* may be described more adequately by recording the conidia as measuring (15)20-48(75-250) μ in length and containing (3)4-10(16-50) cells. The conidia measure 7-7.5 μ in width. Inasmuch as *S. multiplex* becomes a synonym, the complete description of the conidial stage is here included in the following technical description of *Lophiosphaera velata*.

DIAGNOSIS AND POSSIBLE AFFINITY OF THE SPECIES

Perithecia carbonaceous, gregarious, subglobose, being laterally compressed above and exhibiting a linear ostiole, (0.3)0.4–0.6 mm. in length, (0.28)0.35–0.58 mm. in height, (0.15)0.2–0.3 mm. in breadth, endoxylous in origin, for the most part maturing within the wood except for the hysteriform ostiolate crest, ordinarily accompanied by the *Septonema* conidial stage which blackens the wood and often veils the crests of the perithecia; asci 8-spored, clavate-cylindrical, measuring (75)80–90 \times 8–10 μ , interspersed with delicate paraphyses which are finely branched and interwoven above; ascospores hyaline, biseriata, elliptic-fusoid, and slightly curved, (20)22–25(27) \times 3.5–4.5 μ , distinctly equally two-parted with a noticeable constriction at the septum and often with four large guttulae, but potentially 4-celled as manifested at maturity by a less noticeable constriction in each half of the spore and by the presence of three definite septa prior to the germination of the spore.

Pycnidia (seen only in cultures) of the *Plenodomus*—*Hysterophycnis* type, subcarbonous, spherical to conic, ostiolate, minute, measuring (60) 70–85 μ in diameter and (75) 85–105 μ in height, and having a complete wall which is spore-bearing in both its lateral and basal portions; pycnidiospores hyaline, ovate to inequilateral or elliptic oblong, 1.5–2 \times 1–1.5 μ , produced singly at the tips of simple, clavate, closely clustered spore-bearing cells.

Conidia (*Septonema multiplex* B. & C., emended; excl. *S. Mollerianum* Roumeguère, 1887) fuscous, smooth, catenulate, developing by budding at the apex, (5.5) 6–7.5 (8) μ in diameter but variable in length—(14) 24–54 (88, 150, 175, 250) μ —and in the number—(3) 5–11 (18, 30, 35, 50)—of component cells, the cells averaging slightly less than 5 μ in length; conidial filaments generally unbranched, averaging 3–5 conidia, produced terminally on short hyphae or on short conidiophores from the cells of the branched matrical hyphae, the complex forming a loose, effused, black weft on the surface of the wood.

The present records point to a southern range of the species; besides, they suggest that the decorticated wood of *Quercus* is the most favorable substratum for the fungus. The evidence is sufficient to indicate that the records for Louisiana (Ellis and Everhart, 1892), Alabama (Underwood and Earle, 1897), South Carolina (Berkeley, 1874), and the present ones for Florida and Georgia stand for a single species, and that the report of *S. multiplex* by Cooke and Harkness (1881) as occurring on the bark of *Eucalyptus* in California needs verification.

The endoxylous origin of the perithecia and their development within the wood are the features which necessitate the classification of the species with the Lophiostomaceae. The close resemblance in macroscopic features between its fructifications and those of *Lophiosphaera congregatum* Hark. and the resemblance of its mature ascospores to those of *Lophiotrema hysterooides*

(Ellis & Lang.) Berl., a southern species of the same habitat, are to the writer the indications of the most probable specific affinities of the organism. It is the retarded septation of the ascospore, a condition also known for *Lophiosphaera viticola* Sacc., which makes generic disposition of the species difficult.

Now that we know with certainty that the Hysteriaceae are of pyrenomycetous affinity, because connections have been definitely established between species of this family and forms of the Dematiaceae, of the Hyphomycetes, *Lophiosphaera velata* and the forms of the Lophiostomaceae resembling it might well be considered as species transitional to the two families, if the phylogenetic relationship be viewed as a close one.

SUMMARY

1. This paper presents a cultural and taxonomic study of an ascomycetous fungus known heretofore as *Glonium velatum* E. & E. of the Hysteriaceae.

2. Some of the more important features of the development of the fungus in culture are mentioned, and the characteristics of its perithecial, pycnidial, and hyphomycetous stages are described and discussed.

3. For reasons given in the paper, the species is transferred to the Lophiostomaceae and referred to as *Lophiosphaera velata* (E. & E.), a new combination.

4. The pycnidial stage of the species, known only as it developed in culture, is suggestive of the form genera *Plenodomus* and *Hysteropezizis*.

5. The so-called *Dendryphium* stage of *Glonium velatum* is shown to be the hyphomycetous stage of this species and to be identical with *Septonema multiplex* B. & C.

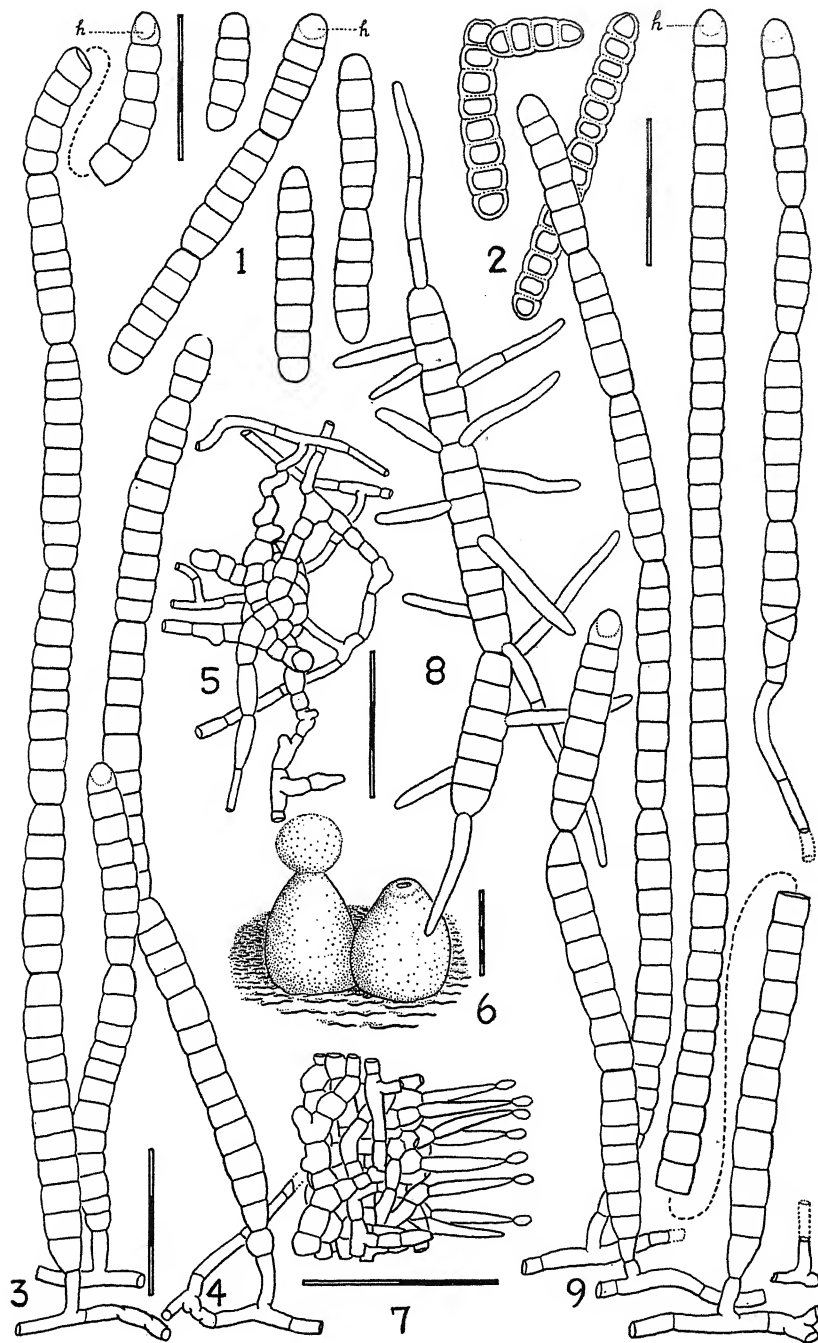
6. The study reveals that the maturation of the conidia in this *Septonema* stage proceeds from the base to the apex of the filament which is produced by the budding and single division of each successive apical cell.

7. This species is suggestive of a probable close relationship between the Hysteriaceae and the Lophiostomaceae.

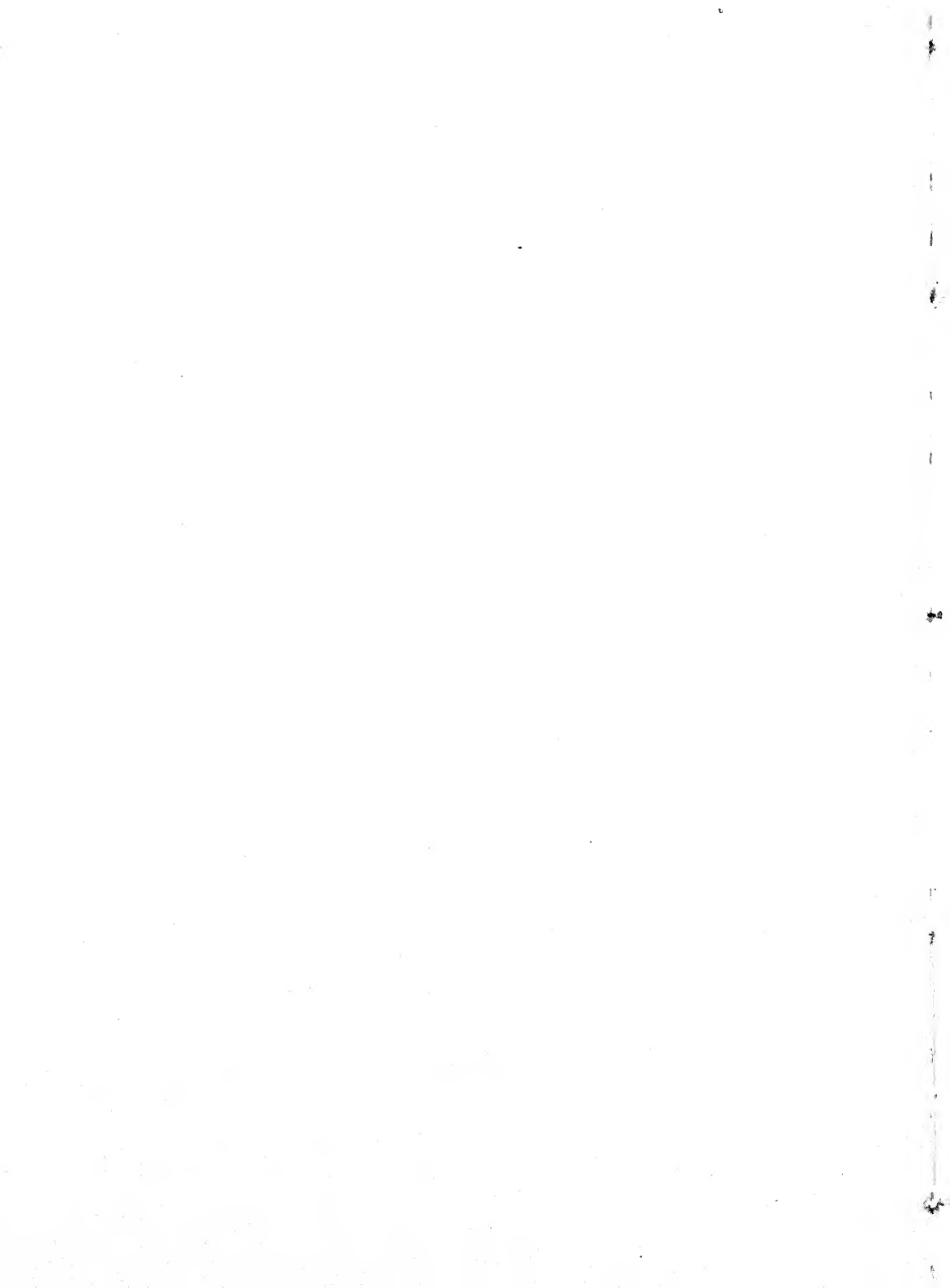
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LOHMAN: *LOPHIOSPHAERA VELATA*



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EXPLANATION OF PLATE I

Illustrating by outline drawings the dark brown *Septonema* conidia (fig. 1-4 and 8-9, *h* indicating the less deeply colored portion of apical cells) and the diagnostic features of the pycnidium (fig. 5-7) of *Lophiosphaera velata*. All of the drawings were outlined with the aid of the camera lucida, and unless otherwise indicated, they represent as reproduced an approximate magnification of 665 \times . (Divisions on all of the scales correspond to intervals of 10 μ .)

Fig. 1-3. Conidia and conidial filaments typical of collection 2751 of Curtis, the type collection of *Septonema multiplex* B. & C. In figure 2 the conidia are shown in sectional view to illustrate relative thickness of the wall.

Fig. 4. A conidial filament from the type collection, Langlois 2220, of the perithecial stage of this species.

Fig. 5-7. Illustrating the important features of the pycnidial stage of the species as obtained in laboratory cultures; figure 6, about $\times 155$; figure 7, about $\times 1335$.

Fig. 8. Illustrating the manner of germination of the *Septonema* conidia.

Fig. 9. Types of conidial filaments obtained in each of several mono-ascospore cultures of this fungus. In connection with the variation of the conidia with respect to size and number of cells, see also text figure 3.

INHERITANCE OF SEX IN CERTAIN SEED PLANTS¹

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INTRODUCTION

The fact that plants have sex is common knowledge, and almost everyone knows of the importance of sex from the standpoint of plant breeding. Most plant breeders, however, have not become highly interested in this important character; so it is not being studied as much as some morphological characters of our common economic plants. The chief reason for this, I presume, is that most of our economic plants are either hermaphroditic or monoecious—that is, they have both sexes on one plant.

Sex is a character, therefore, that cannot be studied from a Mendelian standpoint in these plants. In the dioecious plants, however, we have a different situation, since sex is an important characteristic and often an economic one. For example, in hops, the "male" plant is of little value from the standpoint of producing the resins which are the economic part of the plant. These resins are obtained chiefly from the lupulin, which is a granular secretion in the female inflorescence and the bracts of the female inflorescence, and it is doubtful if pollination or fertilization increases the resins. On the other hand, it has been pointed out that the "male" plants of the asparagus are better producers than the "female."

Cook (1914) calls attention to the so-called Russian or Manchurian hemp, grown for seed, which has male plants that are very slender, spindling, and short, and die much earlier than the females.

Many dioecious plants. Few workers realize that there is a large number of plants which fall in this dioecious group. Yampolsky (1923) points out that dioecism, while numerically not as great as hermaphroditism, is of even wider distribution, being represented in practically every order of plants. Schaffner (1927a) has listed many dioecious plants. The following list collected from various sources in the literature includes a large number of seed plants which have this dioecious characteristic. It must be remembered that not all of these plants are strictly dioecious—that is, some of them exhibit monoecious characteristics to a certain degree. The plants are grouped in the following classes of economic importance: vegetable and field crops, shrubs, trees, weeds, and those herbs not listed as crop plants or weeds. The plants, with both common and scientific names, are as follows:

¹ Published as Technical Paper No. 195 with the approval of the Director of the Oregon Agricultural Experiment Station.

TABLE I. *Seed plants which have been classified as dioecious**Vegetable and field crops*

Asparagus officinalis L.—Asparagus
 Bulbils dactyloides (Nutt.) Raf.—Buffalo-grass or Early Mesquite
 Cannabis sativa L.—Hemp or Red-root
 Humulus lupulus L.—Hop
 Sagittaria latifolia Willd.—Tule Potato (Imperfectly dioecious)
 Spinacia oleracea L.—Spinach

Shrubs

Baccharis halimifolia L.—Groundsel-bush or Pencil-tree
 Myrica gale L.—Sweet Gale
 Ptelea trifoliata L.—Three-leaved Hop-tree or Shrubby Trefoil
 Rhus glabra L.—Smooth Sumac (Imperfectly)
 Schmaltzia crenata (Mill.)—Sweet-scented Sumac (Imperfectly)
 Taxus canadensis Marsh.—American Yew or Ground Hemlock

Trees

Acer negundo L.—Box Elder
 Acer platanoides L.—Norway Maple
 Acer saccharinum L.—Silver Maple
 Ailanthus glandulosa Desf.—Tree of Heaven or Ailanthus
 Carica papaya L.—Melon Tree or Papaya
 Cycas revoluta Thumb.—Sago Palm
 Diospyros virginiana L.—Persimmon, Date-plum or Lotus-tree
 Fraxinus americana L.—White Ash or Cane Ash
 Gymnocladus dioica (L.) Koch—Kentucky Coffee Tree
 Morus alba L.—White Mulberry
 Morus rubra L.—Red Mulberry
 Papyrius papyrifera (L.) Kuntzo—Paper Mulberry
 Phoenix dactylifera—Date Palm
 Populus alba L.—White or Silver-leaf Poplar
 Populus acuminata Rydberg—Black Cottonwood
 Populus angustifolia James—Narrow-leaved Cottonwood
 Populus balsamifera L.—Balsam or Carolina Poplar
 Populus candicans Ait.—Balm of Gilead
 Populus deltoides Marsh.—Cottonwood or Necklace Poplar
 Populus grandidentata Michx.—Large-toothed Aspen
 Populus heterophylla L.—Swamp or Downy Poplar
 Populus italica Moench.—Lombardy Poplar
 Populus nigra L.—Black Poplar or Willow Poplar
 Populus sargentii Dode.—Western Cottonwood
 Populus tremuloides Michx.—American Aspen or Quiver-leaf
 Salix nigra Marshall—Black Willow
 S. vallicola (Dudley) Britton—Dudley's Willow
 S. wrightii Anderson—Wright's Willow
 S. longipes Anderson—Ward's Willow
 S. laevigata Bebb—California Black Willow
 S. toumeyii Britton—Toumey's Willow
 S. amygdaloides Anderson—Peach-leaved Willow
 S. lucida Muhlenberg—Shining Willow

S. lasiandra Benth—Western Black Willow
S. lyallii (Sargent) Heller—Lyall's Willow
S. fragilis L.—Crack Willow
S. alba L.—White Willow
S. baylonica L.—Weeping Willow
S. interior rowlee—Sandbar Willow
S. exigua Nuttall—Slender Willow
S. sessilifolia Nuttall—Silver-leaved Willow
S. mackenziana Barrett—Mackenzie's Willow
S. missouriensis Bebb—Missouri Willow
S. lasiolepis Benth—California White Willow
S. balsamifera (Hooker) Barrett—Balsam Willow
S. amplifolia Coville—Large-leaved Alaskan Willow
S. hookeriana Barrett—Hooker's Willow
S. viminalis L.—Osier Willow
S. taxifolia Humboldt, Bonpland and Kunth—Yew-leaved Willow
S. sitchensis Sanson—Satin Willow
S. bebbiana Sargent—Bebb's Willow
S. bakeri von Seemen—Baker's Willow
S. discolor Muhlenberg—Glaucous Willow
S. scouleriana Barrett—Scouler's willow
S. alamensis (Anderson) Coville—Felt-leaf Willow
S. purpurea L.—Purple Willow
Sassafras sassafras (L.) Karst.—Sassafras Tree
Zanthoxylum americanum Mill.—Prickly Ash (Imperfectly dioecious)
Z. clava-herculis (L.)—Southern Prickly Ash

Weeds

Acnida tamariscina (Nutt.) Wood—Western Pigweed
Anaphalis margaritacea (L.) Benth. and Hook—Pearly or Large-flowered Everlasting
Antennaria neglecta, Greene—Field Cat's-Foot
Antennaria plantaginifolia (L.) Richards—Plantain-leaf Everlasting
Carduus arvensis (L.) Scop.—Canada Thistle
Distichlis spicata (L.) Greene—Salt Grass
Elodea angustifolia (Muhl.) Britton—Narrow-leaved Water-weed
Elodea canadensis (Michx.) Britton—Water-weed
Elodea minor (Engelm.) Small.—Lesser Water-weed
Elodea nuttalli (Planch.) Rydb.—Nuttall's Water-weed
Lychnis alba Mill.—White Campion or Evening Lychnis
Lychnis dioica L.—Red Campion or Red Bird's-eye
Rumex acetosella L.—Red Sorrel or Sour Dock
Smilax glauca Walt.—Saw Brier

Herbs

Arisaema triphyllum (L.) Torr.—Jack-in-the-Pulpit or Indian Turnip
Aruncus sylvestris Kost.—Goatsbeard
Bryonia dioica Jacq.—Bryony
Chamaelirium luteum (L.) A. Gray—Blazing Star
Dioscorea villosa L.—Wild Yam-root
Humulus japonicus Sieb and Zucc.—Japanese Hop
Hydrocharis morsus-ranae—Frog's Bit
Menispermum canadense L.—Canada Moonseed

Mercurialis annua L.—Deep Mercury
Mercurialis perennis L.—Herb Mercury
Napaea dioica L.—Glade Mallow
Sagittaria montevidensis—Arrowhead
Smilax herbacea L.—Carrion-flower or Jacob's Ladder
Smilax hispida Muhl.—Hispid Greenbrier or Bristly Sarsaparilla
Thalictrum dasycarpum Fish. and Lall.—Purplish Meadow Rue
Thalictrum dioicum L.—Early Meadow Rue
Thalictrum L. spp.—Meadow Rue—(Some with bisporangiate flowers)
Tumboa bainesii or *Welwitschia*
Vallisneria spiralis L.—Tape-grass or Eel-grass

REVIEW OF LITERATURE

Phylogeny. There has been a difference of opinion as to whether dioecious plants came from hermaphrodites originally or whether dioecious plants are the original form and monoecious and hermaphrodite the derived. Most writers seem to believe that hermaphroditism is the primary type and dioeciousness the derived. In this latter group are Stout (1923) and Correns (see Jordan, 1908), who believe the dioecious condition to arise in consequence of the physiological or morphological disappearance of one or the other set of members of the hermaphroditic condition. Detjen (1917) describes a grape that is self-fertile, being probably a staminate vine whose long suppressed pistils have suddenly been regenerated and have recovered the power to function. He believes that the prototype of our present-day *rotundifolia* vine, which has two types of flowers, staminate and hermaphrodite, was a true and functioning hermaphrodite. Schaffner (1918) traces the development of sexual dimorphism from its lowest to its highest form, putting dioecious plants at the top. Bond (1915), however, believes that hermaphroditism arose from monoecism or dioecism. He conceives of the hermaphrodite flower as a sex chimaera built up on a clinal or central female basis with periclinal male accessory organs.

Workers not agreed. Many workers who have studied the important characteristic of sex in the higher plants may be placed into two groups: those who are of the opinion that sex is hereditary and determined by either genes (factors) or specific chromosomes, and those who believe that sex is physiological and dependent upon environmental conditions. In the group which believes that sex is Mendelian in determination and that sex expression, while probably complicated, is fundamentally no different from the expression of any other character, there are Bateson, Castle, Correns, Emerson, G. H. Shull, Winge, and others.

Shull (1910b, 1911, 1912), who has done much genetic analysis (1910a), says that "sex is at least predominantly dependent upon the genotypic nature of the individual," and thinks that female plants partially changed to the other sex and self-pollinated would produce only female offspring unless the genotypic nature had been changed. Rosa (1925) holds that environmental influences seem to have no effect in determining sex in spinach and says there

is evidence that differences in sex are due to genetic factors. Sharp (1927) believes that sex inheritance is Mendelian in nature, although capable of modification by environment. Emerson (1924) maintains that sex characters differ in no essential way from other organic characters, either in mode of inheritance or manner of development, and considers it not unlikely that functional dimorphism may exist even where no morphological differences in the chromosome are seen.

Chromosome differences. One point put forth by some is that in several species of dioecious plants, a chromosome difference, either in size or number, has been found between the males and the females. In *Sphaerocarpos* (a lower form) Allen (1917) found a large chromosome in the female cells with a small mate in the male cells. Schacke (1919) observed the same condition in another species of *Sphaerocarpos*. Blackburn (1925) found one chromosome in the male larger than any present in the female of *Lychnis* (catchfly). Darling (1909) thought he could detect some inequality in the distribution of the chromatin to the daughter cells at the reduction division of the pollen mother cell of *Acer negundo*. Kihara and Ono (1925) found 15 chromosomes in male plants of *Rumex acetosa* and 14 in the female. Two of the male chromosomes together are thought to constitute a y -element. Santos (1923) found an unequal pair of chromosomes in somatic cells of *Elodea* (water plant), which go to different daughter cells at reduction. Winge (1914) found x - and y -chromosomes in *Humulus lupulus*, *H. Japonicus*, and *Melandrium album*, and an odd number of chromosomes in *Vallisneria spiralis* males. Winge expresses a belief that "sex chromosomes are found altogether throughout the whole of the vegetable kingdom in dioecious species," although they may not always be demonstrable, as observed by McPhee (1924b), who found no evidence of a sex chromosome in hemp, and Sykes (1909), who could detect no differences in the nuclei of the two sexes of *Hydrocharis morsus-ranai*, *Bryonia dioica*, *Lychnis dioica*, *Mercurialis perennis*, *Sagittaria montevidensis*, or *Cucurbita pepo*. Mottier (1907) published a detailed account of meiosis and mitosis in *Podophyllum*, a member of the barberry family, but made no mention of sex chromosomes. Indeed, Schaffner (1927c) points out that 150,000 species of heterosporous plants have unisexual gametophytes but no sex chromosomes, or "allosomes" as he calls them (1924). Even autosomes have attraction for their allelomorphic mates at synapsis, and Schaffner (1925a) considers this a sexual phenomenon. Consequently, he thinks autosomes are just as much sex chromosomes as those to which some attribute the responsibility for the determination of sex. There frequently are differences between male and female plants that indicate an allosome linkage similar to that found in animals. Allen (1923) found that a *Sphaerocarpos* character of having the spores separate in the tetrads instead of united was sex-linked, and transmissible by only the female gametophyte. Bateson and Sutton (1919) concluded after a study of *Begonia* that doubleness of flower is sex-linked. This allosome linkage points to definite factors for sex characters located on definite chromosomes.

Opinions differ as to just how the factors for sex are carried by the chromosomes. Bateson and Punnett (1908) expressed a belief that one and not both sexes would be found to be heterozygous for sex. Castle (1909) believes femaleness depends on a factor wanting in the male and that the female is the male condition plus something else. Jordan (1908) states that Correns considers each germ cell has originally a fixed sex tendency; that germ cells of one sex have one and the same sex tendency, those of the other partly the one, partly the other; and that a primarily fixed differentiation in the developmental vigor of the germ cells with different sex tendencies that unite at fertilization brings about a decision favorable to one or the other sex. Doncaster (1915) subscribes to the idea of a chromosome balance for sex more or less easily upset. Emerson (1924) says that "sex is probably an expression of the interaction of several, perhaps many, factors located on different chromosomes."

Sex determined by environment. Among those who believe that sex is determined by environment are Schaffner (1921b, 1923d, etc.), Stout (1923), Yampolsky (1916), and Riddle (1927). They do not think that every character is Mendelian in determination. Schaffner (1915) states that all protoplasmic structures have hereditary factors, although all normal Mendelian heredity must have its factors in the chromosomes alone. Sexuality is a general potentiality, and the balance may swing to maleness or femaleness through the influence of environment or a physiological state which is brought about through a physiological gradient in the regular ontogeny. They maintain that if there is a difference in the chromosomes, it is a result of sex rather than a determiner of it (Schaffner, 1925c). They point to sex reversals, in which a male plant changes to female, or vice versa, as conclusive evidence that sex is not determined by genes or chromosomes. Such sex reversals were long ago noted. Burbridge (1890) said, "It has long been known that dioecious plants now and then alter their function, those formerly males producing female flowers or vice versa." Schaffner (1919) was able to get reversals in hemp from femaleness to maleness of every degree of intensity or completeness, both in number of flowers produced and in the degree of perfection of the sexual structure involved, simply by growing the plants out of season with a deficiency of light and a shallow soil heated partly from below. The main factor responsible for this confusion of sexual state he held to be the relative length of the daylight period. The action of the light is thought to go deeper than merely reducing the food supply by reducing photosynthesis, it being conceivable that there may be a direct effect on the ultimate chemical and electrical activities of the cell (1923b).

Later work of Schaffner (1925b) has shown that even the very possibility of reversal depends to a considerable extent on whether the plant is drawing the proper nutrients, especially nitrogen from the substratum at the time, high soil nitrogen favoring reversal. Halsted (1899) observed a greater percentage of pistillate flowers as soil depth increased. Fumigation by to-

bacco smoke reversed the sex of carpellate plants (Schaffner, 1928). Mixed sexual expression or sex reversal was obtained with more than 75 per cent of hemp plants grown under abnormal conditions by Schaffner (1922a). Later he got 100 per cent with better knowledge of environmental control. Proper short-light-temperature periodicity makes all plants bisexual, Schaffner (1930) has recently concluded. Pritchard (1916) altered the sex of some hemp plants (both sexes) by removing flowers, and believes that if the proper stimulus were used, pistil formation could be induced in all the males.

McPhee (1924a), however, tried many different kinds of stimulation in attempts to change sex but was not successful. Hirata (1927) explains sex reversals and intersexes in hemp on a Mendelian basis. He believes in a gene-balance for sex brought about by differing valency of the x - and the y -chromosome. The factors for sex located on these chromosomes are thought to produce enzymes which determine sex and which are influenced to a varying extent, depending on the sex, by differing environments.

Hemp has been the subject of a great deal of study in regard to its sexual behavior. Malloch (1922) points out its desirability for sex work. Prain as early as 1904 pointed out the many unusual hemp plants from the standpoint of sex.

Sex in *Arisaema* has been reversed at will by Schaffner (1922a) by changing the conditions. Unfavorable, dry conditions favored maleness, while rich, wet conditions caused femaleness. By carefully controlling the food supply, he was able to cause twin *Arisaema triphyllum*, which are normally of the same sex and were originally in this case, to become of opposite sex, even after they had been developed as blooming plants and while they were still connected by a considerable band of living tissue (1922c, 1926c). Japanese hops planted in the greenhouse in winter became greatly dwarfed and the sexual state much confused in a very large percentage of cases (1923a). Sex of *Thalictrum dioicum* may be changed, though with difficulty (1925d). Unfavorable conditions seem to favor maleness in most plants, but Anthony (1917) reports that lack of vigor in the plant suppresses stamen development in perfect sorts of strawberries. Still, Gardner (1923) points to much work to show that "femaleness is associated with rich soils, abundant moisture, liberal spacing, the vigor of youth, favorable growing conditions. Maleness, on the other hand, is associated with somewhat less favorable growing conditions."

Schaffner (1920) was not able, however, to induce a monoecious character in buffalo grass, a strictly dioecious plant which has been reported from time to time to be monoecious, probably because two plants from different roots sometimes grow very close together.

Female plants of *Lychnis dioica* have been observed to bear male organs but only when attacked by a certain smut fungus which is able to fruit only in the male organs, according to Blakeslee (1907). No production of female organs took place in male flowers when they became infected (Shull, 1912).

Mason and Lewin (1925) found that as oil-palms grow older, they produce a smaller proportion of female flowers, apparently because of exhaustion of reserves.

Meehan (1870) contended that "with a weakened vitality comes an increased power to bear male flowers, and only under the highest conditions of vegetative vigor are female flowers produced."

Robbins and Jones (1925) believe that all asparagus flowers are potentially hermaphrodites, but that during floral development there is, except in rare cases, an abortion of one set of sex organs.

Stout (1919) states that maleness and femaleness are quantitative differences with all grades of intersexes, subject to modification or even complete determination by processes of growth, development, and interaction of tissues. He believes that "much variation in maleness and femaleness exists in sex organs that are morphologically perfect." Also he (1923) is of the opinion that morphological differentiations of sex are fundamentally an extension of the phenomena of somatic differentiations, and are not determined by sex chromosomes.

Yampolsky (1916, 1919, 1920a, 1920b), working with dog mercury, concluded that sex is not a fixed condition and is subject to reversal. He found an almost complete series of flowers intergrading from male to female, and explains this intergradation by a doctrine of varying potency for sex in germ cells.

Valleau (1916), however, working with the grape, which has male, female, and hermaphroditic plants, concluded that sex is Mendelian and that there are suppressors of the factors for maleness and femaleness. This suppressor idea would account for the differing degrees of sexuality observed by Schaffner, Stout, and Yampolsky.

It is interesting in connection with sex reversal in plants to note that Russo, according to Jordan (1910), produced rabbit offspring almost entirely female by administering lecithin to pregnant rabbit mothers.

Another point advanced by Schaffner (1927b, 1927c), who believes that sex is determined in the egg before fertilization and eggs of one sex attract sperms with the allosome (if there is one) while eggs of the other sex attract sperms without an allosome, is that "in the vast majority of types of species of plants and animals, the time of sex determination or of sex reversal does not correspond with the shifting of the chromosomes." Sex determined at the time of fertilization, which results in dioecious plants, is no more intense or permanent than sex determined at any one of the other 11 periods in the life cycle at which he says it may be determined (1923c). Also he (1923b) states that "sex reversal and changes in the sexual state can be brought about as easily, if not more so, in the dioecious sporophyte as in the monoecious."

In lower forms of plant life the dioecious condition is also common, and so they are often used as illustrations. Schaffner (1927d) points out that in

Spirogyra each of two conjugating filaments may react in one part as female and in another as male toward the other, and these filaments are haploid and come from the same reduction spore. In *Oedogonium*, he says, "the same balance of genes has produced practically every kind of sex reaction known." Sister cells, one plus and the other minus, from a haploid source may fuse, as in the egg cell and ventral canal cell of gymnosperms, and in the two polar nuclei of various seed plants which fuse with each other sometimes before fusing with the sperm nucleus. Wuist (1913) found that the ostrich fern develops male prothallia under conditions of poor nutrition and female under conditions of good nutrition. Blakeslee (1906), however, maintained plus and minus strains of *Phycomyces* and *Mucor mucedo* nonsexually for over 100 generations without apparent change in sexual behavior, and Allen (1919) found sex to be apparently determined at the time of spore formation in *Sphaerocarpos*, as each spore tetrad has two male and two female spores. It should be borne in mind in considering dioecious mosses and liverworts that the dioeciousness applies only to the haploid generation and not to the diploid as it does in dioecious higher plants. Only a small percentage of mosses and liverworts have dioecious gametophytes, some being of various grades of monoeciousness, as pointed out by Collins (1919).

Schaffner (1929a) grew seed from a partially reversed, self-pollinated, staminate tree of *Morus alba* and obtained trees of both sexes and mixed sex, showing, so he says, that genetically the original staminate tree had potentialities for both sexes.

From a study of *Sagittaria*, Schaffner (1929b) found that in patches growing under different conditions the percentage of monoecious, carpellate, and staminate inflorescences varied, indicating the influence of environment on sex. Of individual flowers, numbers of staminate and carpellate ones were nearly equal in different patches, showing that a fluctuating sex-determining and sex-reversal balance works in such a way that approximately equal numbers of female and male floral units are produced. Tiedjens (1928) increased the production of staminate cucumber flowers by increasing light and increased the number of pistillate flowers by decreasing light, but finds these changes to be due to changes from one or the other type of flower to leaf and stem tissue. He concludes that environment does not, therefore, determine the sex but merely produces conditions which make possible the expression of potentialities in the plant. In some plants there are perfect flowers, and imperfect flowers of one sex, but not the other. Harris (1915) found a far higher coefficient of variation for number of pistillate flowers of aroids than for number of staminate flowers. Cook (1912) describes two types of pomegranate flowers, one perfect, the other staminate.

Schaffner (1926a) points out a weakness in the arguments of those who seek to explain sex as entirely Mendelian: "What is the cause or factorial mechanism, if any, that determines the sex of a specific region in diploid and haploid hermaphrodites and in diploid and haploid, bisporangiate sporo-

phytes?" Emerson (1924), however, asks in turn, "Is the association of pistils and stamens in a single flower either more or less mysterious than the differentiation of the aleurone layer from the rest of the endosperm?" The differentiation of the aleurone layer is not supposed to be due to a new "balance" of genes or a new alignment of chromosomes. The physiological conditions determine one reaction in one layer and a different one in another with the same balance of genes.

From time to time other ideas on sex determination have been advanced that differ from the Mendelian view but might be construed to agree somewhat with the environmentalist idea. In 1872-1878, according to Wester (1914), Ciesielski claimed that fresh pollen gives male seeds and stale pollen female seeds, and presented considerable experimental evidence to back up his conclusion. In 1909 an English physician advanced the idea that sex in man is determined by which ovary the egg came from (Jordan, 1909). Johnson (1914) published a history of the discovery of sexuality in plants.

As to the direct, immediate cause of sex, several investigators support the theory of varying rates of cell metabolism. Camp (1929) found greater catalase activity in tissues related to staminate structures than in tissues related to female structures. Jordan (1910) inclines to the view that female-ness is caused by dominating cell-anabolism and maleness by preponderant cell-katabolism. Satina, Demerec, and Blakeslee (1926) found significant average differences in catalase content between male and female tissue. Satina and Demerec (1925) and Satina, Demerec, and Blakeslee (1926, 1927) showed the existence of biochemical differences between male and female by use of Manoilov's reaction and KMnO_4 . Both gave results about 85 per cent accurate, but not identical, though closely parallel, showing both to detect reducing substances, but not entirely the same ones. Both reactions depend upon quantitative rather than qualitative differences, they state. Schaffner (1922b) believes that sexual conditions are probably positive and negative states of atoms or molecules contained in the living cell.

One theory which is upheld by a good many investigators is the quantitative theory of sex determination. Riddle (1927), one of those who first supported it, gives the essential parts of it as: that prospectively male gametes have a higher metabolic rate than females, as do male embryos and adults; that these metabolic differences can override the normally controlling influence of the chromosomes; that sex chromosomes or genes probably normally determine sex through controlling metabolic rate; that intersexes and hermaphrodites can arise from chromosomal or genic causes but can also arise from a metabolic cause while chromosomes and genes are normal; and that the metabolic distinction found cannot be interpreted as a secondary sex character.

Schaffner's studies of monoecious plants (1921a) which show a change from one sex to the other, either in the main stem or in side branches, have shown that changes from carpellate structures in the lower part of the in-

florescence to the staminate in the upper part are apparently more common than the opposite condition. Thus, as the monoecious plants get older, we find them changing from female to male, just as do many dioecious plants. Indian corn (*Zea mays*) is a good example of this, ears coming out on the lower part of the stalk and the tassel at the very top. *Typha* (cat-tail) ordinarily has an inflorescence carpellate below and staminate above, but sometimes may have several alternating segments with alternating sexual states (Schaffner, 1926b). Fugii (1895) obtained sex reversal from male to female on *Pinus densiflora* by increasing nourishment of branches; and Higgins (1916) reports that staminate papaya or melon trees occasionally change sex completely when their tops are cut off, whereas as yet no pistillate tree has ever been known to change its sex.

The writer (1931a) has previously pointed out that more than likely both groups are correct, at least in part. Undoubtedly the germ plasm has much to do with the determination of sex, but maybe it does not decide all in regard to this character. Also he (1931b) has shown the possibilities in developing new varieties of hops. These new varieties may be less susceptible to sex reversals.

In some plants sex appears to be a very delicate balance and may be thrown one way or another. As Emerson (1924) says, "In many dioecious plants and perhaps in some animals the genetic balance is so delicate that the reaction may go one way in one environment and the other way under other surrounding conditions with the occurrence of various sex intergrades when environment is less extreme or less constant." The expression of many other characters, as well as sex, depends upon environment, he points out. Schaffner says, "Of course, one must not confuse sex characters with sexual states and every character whether a sex character or not has some kind of potentiality back of it."

Morgan (1932) indicates the importance of both inheritance and environment in sex determination and tells of the attempts during the last 32 years to isolate (in a genetic sense) the factors that determine sex.

EXPERIMENTS AND RESULTS

A worker with dioecious plants is very early convinced that in plants we have several conditions or degrees of sex. One worker, Correns, has gone so far as to recognize at least thirty different kinds and Schulz has listed ten distinct forms of ash. In hops (*Humulus*), the dioecious plant with which the writer is most familiar, there are four types of plants which are fairly common. There are plants which are neuter (flowers reduced and sterile), some that are strictly male, others that are strictly female, and a few monoecious plants which exhibit both sexes. In examining literally hundreds of plants which exhibit this condition of both sexes, the writer has observed but two plants which were primarily female and which had later shown the male

sex. In all other cases it has been the male plant which has reversed to the female condition. This is opposite to the sex reversals obtained with many other seed plants. Of course, from a functional standpoint there are but three types—male, female, and neuter.



Fig. 1, 2. Fig. 1 (left). Typical male flowers of *Humulus lupulus*. Fig. 2 (right). Typical female flowers of *Humulus lupulus*.

In 1930 outstanding male plants of hops (*Humulus lupulus*) in a grower's yard were staked so that root cuttings could be obtained the following spring for planting in our experimental yard. These plants showed no signs of sex reversals. Male flowers are shown in figure 1 and female flowers in figure 2. The root cuttings, 23 in number, were planted in 1931 in our experimental yard at Corvallis, Oregon, and much to the writer's surprise practically all of these plants exhibited a monoecious condition; that is, they produced female flowers as well as male flowers. Estimates of the percentage of female flowers were recorded.

In the spring of 1932 additional roots were obtained from the original source. There were 98 male plants in this group, making a total of 121 male plants observed for sex reversals. The estimated percentage of female flowers on these plants is given in table 2.

TABLE 2. *Estimated percentage of female flowers on 121 "male" plants in experimental yard, Corvallis, Oregon, 1931 and 1932*

Male plant no.	1931	1932
	Per cent of female flowers	Per cent of female flowers
1	10	0
2	0	0
3	50	1
4	3	20
5	65	5
6	5	Trace
7	7	15
8	70	Trace
9	75	Trace
10	5	Trace
11	40	Trace
12	60	Trace
13	75	1
14	15	1
15	75	Trace
16	1	2
17	No bloom	Trace
18	" "	Trace
19	" "	15
20	50	Trace
21	60	0
22	50	Trace
23	75	Trace
24		Trace
25		1
26		0
27		Trace
28		1
29		0
30		Trace
31		1
32		Trace
33		0
34		1
35		0
36		0
37		0
38		2
39		0
40		0
41		0
42		0
43		0
44		0
45		0
46		0
47		0
48		0
49		0
50		0
51		0
52		2
53		0
54		Trace
55		0
56		0
57		0
58		0
59		0

TABLE 2—Continued

Male plant no.	1931 Per cent of female flowers	1932 Per cent of female flowers
60		0
61		0
62		0
63		0
64		0
65		0
66		0
67		Trace
68		0
69		0
70		0
71		30
72		0
73		0
74		Trace
75		0
76		0
77		0
78		0
79		0
80		0
81		2
82		2
83		1
84		0
85		0
86		5
87		10
88		0
89		0
90		20
91		0
92		10
93		10
94		0
95		0
96		2
97		0
98		3
99		0
100		0
101		2
102		0
103		10
104		0
105		0
106		0
107		10
108		15
109		2
110		0
111		0
112		3
113		1
114		0
115		0
116		10
117		10
118		0
119		0
120		0
121		7

"Male" flowers on "female" plants. In several years' experience with hops the writer has never seen a typically female plant reverse its sex and produce male flowers. In the experimental yard in 1932 a female plant of the species *Humulus neo-mexicanus* produced a whorl of male flowers and then continued with whorls of female flowers. Figure 3 shows this condition much better than it can be described.

Neuter plants. Neuter (also called bastards and sexless) hop plants (*H. lupulus*) are common in some yards and often are a source of loss to a grower; for even though they usually are the largest and greenest plants in a yard, they produce no cones. For example, one grower reported about 6000 of these neuter (growers call them bastard) plants in a 40-acre yard, or nearly 25 per cent.

These neuter plants have been accounted for in many ways. Some say that they are the result of disease (virus); others state that they are females

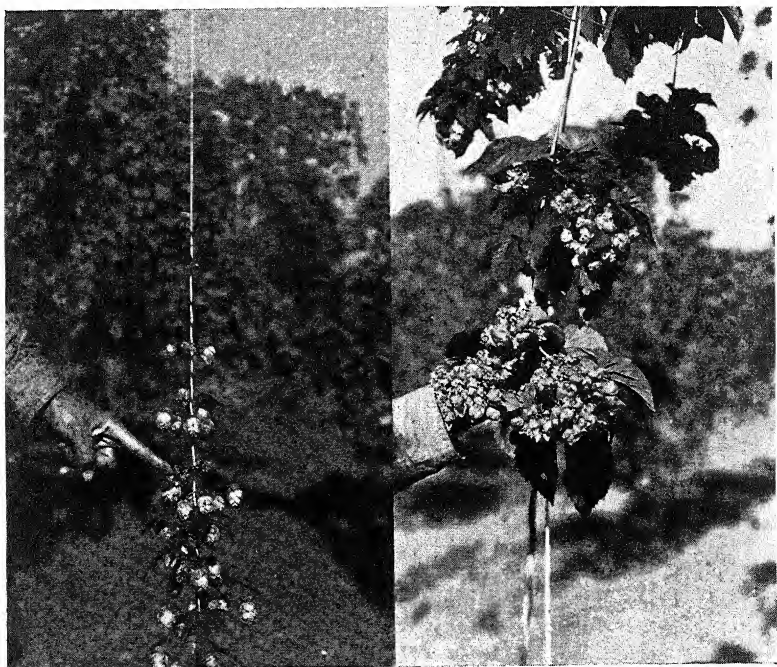


Fig. 3, 4. Fig. 3 (left). Sex reversal in *Humulus neo-mexicanus*. This typical "female" plant produced several whorls of female flowers, one whorl of male flowers, and finally a few whorls of female flowers. Fig. 4 (right). This neuter plant, *Humulus lupulus*, produced no flowers for two years. In 1932 it developed peculiar bunch-like clusters of hermaphroditic flowers.

that have reverted and soon will change to males, etc. None of these theories has been proved. In fact, our observations have not revealed any changes in these neuter plants until this year, when a neuter plant that had been under

observation for three years produced both male and female flowers in peculiar grape-like bunches (fig. 4). This indicates that these plants were not "sex-less" but only neuter for the time being.

One trial to overcome this neuter condition was carried on in 1932. It had been suggested that the trouble may be due to nutrition, so various rare fertilizer elements were applied to individual neuter plants. The results are given in table 3.

TABLE 3. *The effect of various fertilizers on fruiting in neuter hop plants at Corvallis, Oregon, in 1932*

Treatment	Plant no.	Effect	Type of growth
Check—no treatment	1	None	Vigorous
Treble phosphate	2	"	"
" " + sodium borate	3	"	"
" " + copper sulfate	4	"	"
" " + manganous sulfate	5	"	"
" " + potassium iodide	6	"	"
" " + manganese phosphate	7	"	"
" " + sulfur	8	"	"
Check—no treatment	9	"	"

The treble phosphate in each case was applied at the rate of three pounds per hill or a ton per acre. The rare fertilizers were applied per hill as follows: sodium borate, 10 gm.; copper sulfate, 27 gm.; manganous sulfate, 27 gm.; potassium iodide, 5 gm.; manganese phosphate, 27 gm.; and sulfur, 57 gm.

DISCUSSION OF RESULTS

The review of the literature on sex, chiefly in seed plants, reveals differences of opinion regarding the determination of sex. The author makes no attempt to present a complete phylogenetic study, but a few references on some of the lower forms, such as the fungi and ferns, are given. This discussion deals chiefly with sex in the seed plants where this character appears to be more stable.

In hops the most common sex reversal is found on male plants, but a sex reversal on a female plant, *H. neo-mexicanus*, was found in 1932. Hop growers have long observed this phenomenon on male plants, but none that the author has contacted has ever noted a reversal on a female plant. In the 1931 trials, reversals of males to females, indicated by the percentage of female flowers on male plants, varied all the way from 0 to 75 per cent female flowers. In 1932 these same plants showed fewer reversals. In the latter year the percentage of female flowers varied on these plants from 0 up to 20 per cent. It was of interest that the plant which showed the highest reversals in 1932 was one of the lowest in 1931. Plants that had 75 per cent of female flowers in 1931 showed as little as a bare trace, and a plant with 50 per cent of female flowers in 1931 had nothing but male flowers in 1932.

This particular study involved 23 male plants which were grown from cuttings from males that showed no reversals in 1930.

Nearly 100 male plants under observation in 1932 showed some reversals, but far less than observed in the previous year. The reversals varied all the way from 0 to 30 per cent of the male flowers. Sixty-four per cent, or practically two-thirds of the male plants set out in 1932, showed no reversals of female flowers.

Our observations indicate that one cause of these reversals may be improper balance between root and top growth—that is, the cuttings set out in 1931 were just 6-inch underground-stem cuttings from the large established plants in a grower's yard. These root cuttings made a large above-ground growth, as much as 20 feet, in their first season, and produced many flowers. In other words, the plant was out of balance, and disturbed physiological gradients were produced by the improper balance between stem and root. There was an enormous top growth with a small amount of root system, and this appears to be one of the chief causes, as far as the writer can observe, for the large number of reversals in 1931. In growers' yards of established plants there were no more than the normal reversals in 1931. In 1932 there were not so many reversals from plants just set out because the season was not so favorable for hops; the above-ground growth, as a rule, was only about 7 feet in height, and there was not such a lack of balance between root system and top growth in that year. This idea of one of the causes of reversal will be studied further. Probably there was an excess of carbohydrates over nitrates because of the large tops, as suggested by Gardner (1923).

The peculiar and unusual condition of a female plant showing male flowers was observed for the first time this year. This phenomenon is uncommon, and so special note is made of it. The peculiar method of reversion, whereby an entire whorl of flowers is of a different sex, is of interest. This plant is of a different species than the common commercial hop, and as before indicated, belongs to the species *H. neo-mexicanus*. Cuttings will be taken from this plant and set out in 1933, and observed under various conditions to see if this reversion continues. Also, it will be of interest to note if reversions occur on the original plant after it is thoroughly established.

Another condition in the commercial varieties of hops is known as "sexless" or "bastard" plants and now termed neuter plants. These are so common that they are of economic importance. The plants are large and vigorous, but produce only small, abortive flowers. Growers have stated that the usual procedure is substantially as follows: Male plants change to monoecious ones and the latter in turn change to neuter ones. There is no scientific proof for this, and the writer had never observed a neuter plant with any developed flowers until this season, when one neuter plant which had been under observation for three years formed peculiar bunches of flowers consisting of both males and females. Growers' interpretations of these changes, therefore, may be correct. Seeds of this plant are being saved and

will be grown in future years. It is of real importance to determine the cause of this neuter condition, and it may have some bearing on the entire problem of sex in plants.

No one working with dioecious plants in an experimental way and attempting to improve them can do so without noting some peculiar conditions in regard to the character known as sex. Also, no one can help but recognize the importance of both environment and heredity in the determination of this character. The author, having formerly worked practically altogether with monoecious plants, realizes how one could emphasize the importance of heredity in determining sex. But as soon as detailed work was begun with the dioecious type of plant, it could be readily seen that sex is far more complicated than most realize, and that environment plays a rôle. Also, there are possibilities of developing strains of a dioecious plant which do not revert from one sex to the other so quickly, and possibly are not so susceptible to the neuter condition.

Many different opinions have been given in regard to what determines sex, but the writer has not noted the specific idea that sex reversal may be due to mutations in individual cells due possibly to mutable genes. Therefore, late in the spring of 1932 cuttings from plants were exposed to various strengths of x-ray with the hope of determining the effects of the ray upon sex. These plants are growing but did not produce flowers in 1932. This work should be of value not only for studying sex in plants, but also for obtaining information which might throw light on the effect of x-rays upon sex in animals, which, it is claimed, makes neuters in man.

All of this, without a doubt, will have some application in studying sex in animals. Being able to work with large numbers and produce several generations in a short period, there are many advantages for studying sex in plants and applying it to animals in the same way as we do other genetic information. It is time that the geneticists who are interested in things of this type were making more studies to see whether the character of sex falls in the same category as any morphological characters which are being studied. At present there are many phenomena which no one can explain, and when they are explained, they may upset many of the traditional views of sex to which we now hold.

SUMMARY

The literature of sex in seed plants is reviewed, and a table of over one hundred different seed plants that are classified as dioecious is included.

Results of three years of observation and two years of experimental work are given. These show that sex reversals from male to female are fairly common in the cultivated species of hops, *Humulus lupulus*. Cuttings from male plants which showed no sign of sex reversals gave male plants in 1931 which formed a series that ranged all the way from plants exhibiting no sex reversal up to plants which had 75 per cent female flowers. In the following

year the number showing reversals was greatly reduced, as were the percentages of reversals.

In 1932, 98 cuttings from normal male plants were planted. These showed fewer sex reversals than those planted in 1931, but many of them showed a percentage as high as 50.

Reversals of females to males are not common and were not observed until 1932, when one entire whorl of male flowers on a female plant of *Humulus neo-mexicanus* was observed.

Neuter plants are fairly common in the hop fields of western Oregon. One yard reported as many as six thousand in forty acres. They are not only of interest but of economic importance. Growers attribute this condition to several different causes, and these are discussed. In 1932 one neuter plant was found to bear peculiar bunches of both male and female flowers. This is the first plant under control conditions which has produced any fruit. This plant might be a neuter with special genetic constitution. Our seedlings, if we get any, should indicate whether or not this may be the case. A fertilizer trial designed to overcome this neuter condition gave no results.

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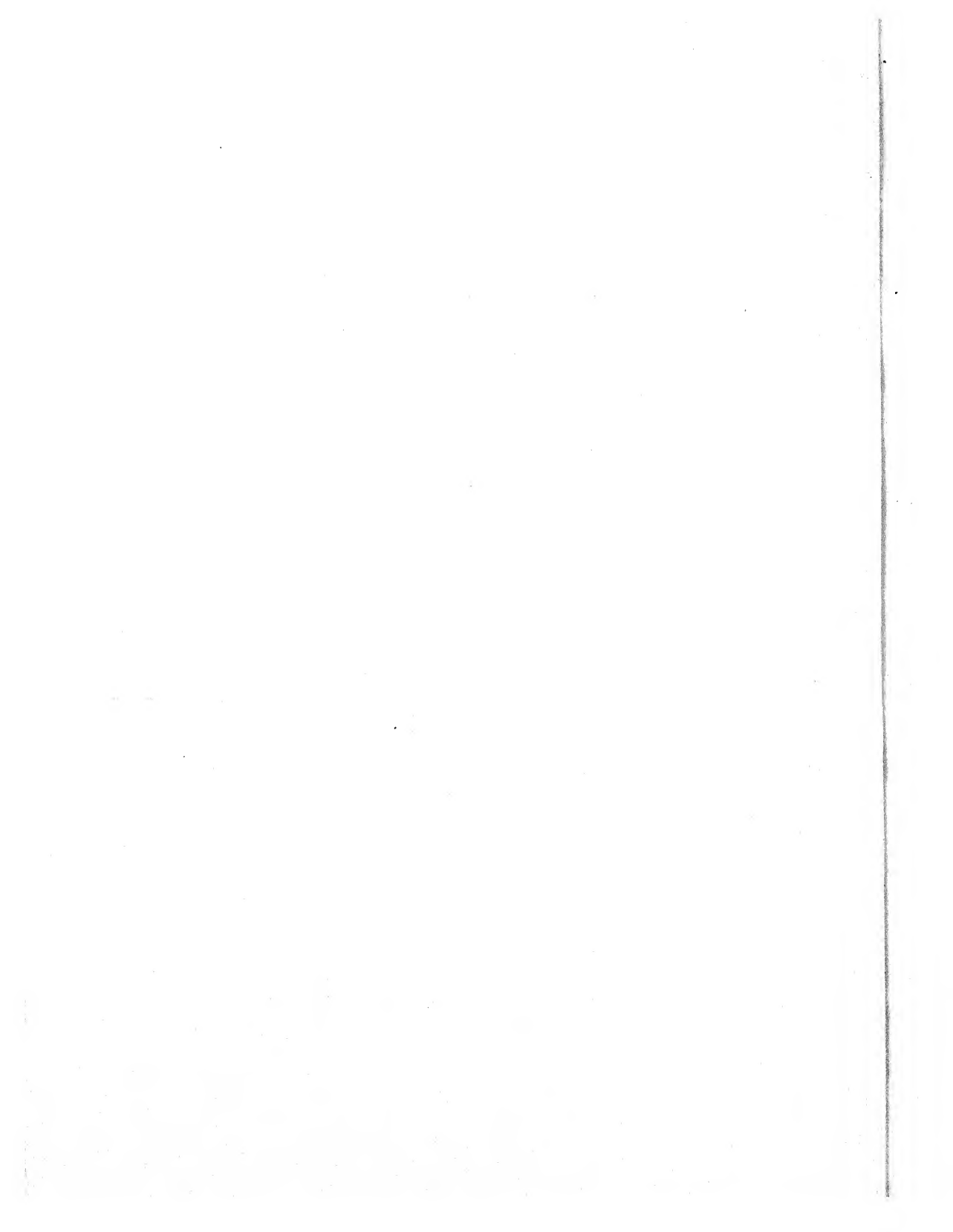
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EFFECT OF EXCESSIVE HUMIDITY ON THE RESISTANCE OF CITRUS PLANT TO SUN SCORCH

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To find out the effect of excessive humidity on the resistance of citrus plants to sun scorch, a moist chamber was designed (fig. 1) in which the plant

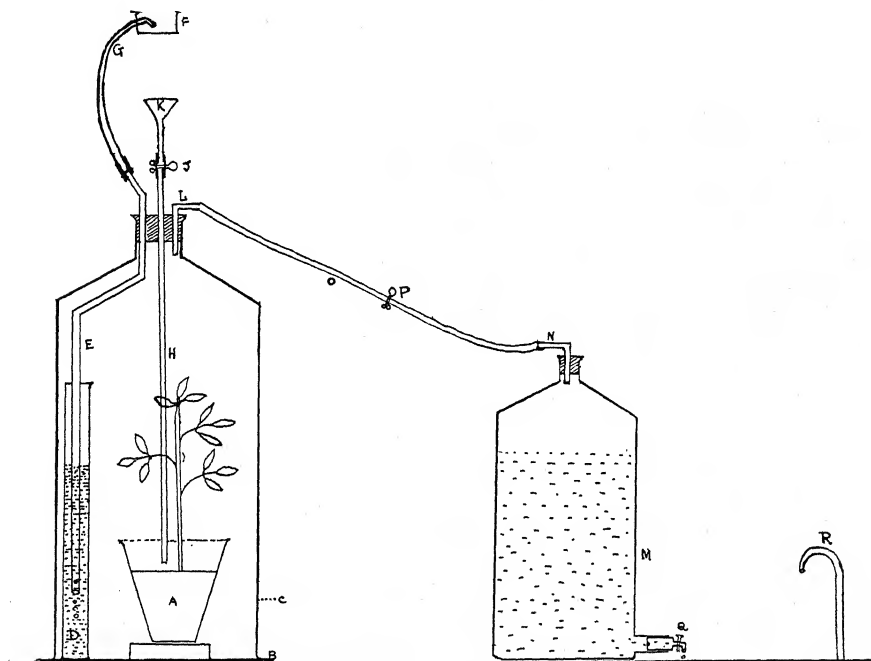


Fig. 1. *A*, Citrus seedling growing in a pot. *B*, Glass plate. *C*, Bell jar. *D*, Trap jar containing sterilized water. *E*, Glass tube dipping into water contained in jar *D*. *F*, Laboratory window. *G*, Rubber tubing connecting the chamber with outside air. *H*, Glass tube for irrigating the pot. *J*, Pinchcock. *K*, Funnel for irrigation. *L*, Glass tube connecting the chamber with the aspirator bottle. *M*, Aspirator bottle. *N*, Rubber tubing connecting the aspirator with the chamber. *P*, Pinchcock. *Q*, Outlet of water in the aspirator bottle. *R*, Water tap.

could continuously be kept under an excessively humid environment well supplied with fresh and pure air. Inside the moist chamber was kept a six-months-old seedling of *Citrus medica* growing in a pot *A* and another seedling

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outside in the room as the control. The pot *A* was placed on a plate of glass *B* and covered with a bell jar *C*. Inside the bell jar was kept a jar *D* containing sterilized water with a glass tube *E* dipping into the water. The tube *E* was connected by means of rubber tubing *G*, the open end of which was exposed to the outside atmosphere through the laboratory window *F*. By means of tube *H*, funnel *K*, and pinchcock *J*, the pot was irrigated with sterilized water whenever required. The outer end of the third tube *L* was connected with an aspirator *M* by means of rubber tubing *O* carrying a pinchcock *P*.

The aspirator bottle was filled with water, and the whole apparatus was made air-tight. As the water was allowed to run out slowly through the outlet *Q* of the aspirator, only fresh air from outside entered the chamber through the open tube *G*, which, passing through the water contained in trap jar *D*, became saturated with moisture. The plant under experiment was thus kept constantly supplied with fresh and moist air free from extraneous matter.

It is clear that the plant *A* was living under conditions of excessive humidity, whereas the control was kept under ordinary conditions of growth. This could easily be judged from the film of condensed moisture, which remained deposited on the inner surface of the bell jar. It was noted that new leaves that developed in the chamber were considerably larger than the normal ones. They had thinner and softer laminae. Their epidermis consisted of thin-walled cells with hardly any cuticle. Some of the older leaves were shed while the seedling *A* was inside the chamber. All these changes were induced by excessively humid conditions in which the plant was kept. As such there was no evaporation from the surface, while the function of absorption by its root system continued. This resulted in vigorous growth of foliage, under conditions unfavorable for the development of cuticle. The control showed no abnormal growth in foliage and no leaf fall. The seedling was taken out of the moist chamber after 40 days, and both the seedlings were kept outside in the sun. In seedling *A* within four hours after this transfer all the leaves which had grown on the plant while in the chamber and also some of the older leaves showed numerous white, dried-up patches, especially along the edges of the leaves (Massachusetts Agr. Exp. Sta. Bull. 170, 1916), (fig. 2, 3). Hand sections showed that these dried-up patches were caused by the collapse of the tissues and disintegration of the chlorophyll. Gradually all the leaves wilted and ultimately the whole plant dried up after twenty-four hours, in spite of being kept well supplied with water. The other seedling kept as the control remained unaffected and showed no signs of wilting under these conditions. This scorching and sudden collapse and the consequent death of the plant was evidently due to excessive evaporation from the general surface of the leaves (Hartig, 1894), in the case of the plant on which the cuticle could not fully develop, and to the failure of the transpiration mechanism and the consequent inability of the plant to adjust itself to the suddenly changed surroundings. These conditions in the seedling *A* were

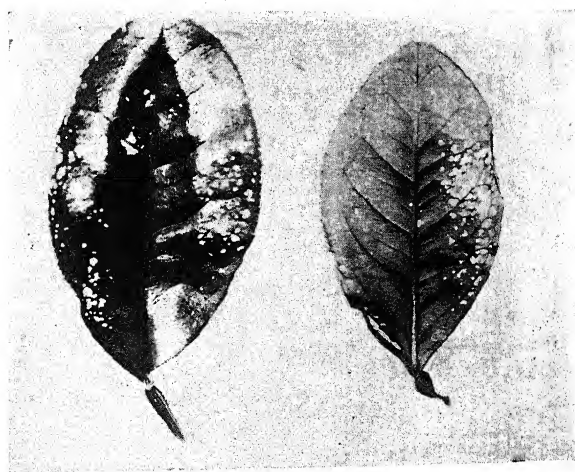
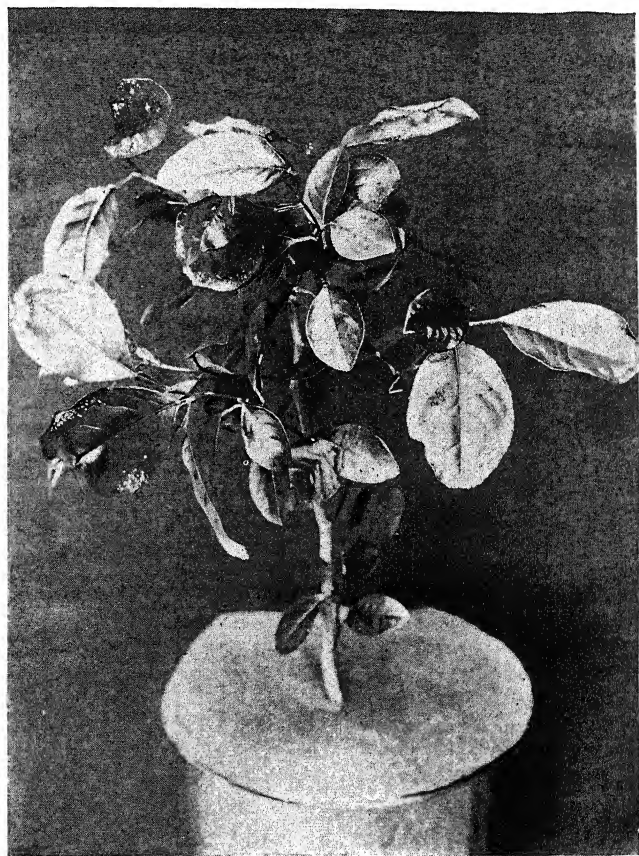


Fig. 2, 3. Fig. 2 (above). Scorched seedling of *Citrus medica* after four hours' exposure to sun. Fig. 3 (below). Scorched *Citrus* leaves,

brought about by its prolonged confinement in a fully saturated atmosphere.

Newcombe and Bowerman (1918) have also recorded the increased growth, thinning of leaves, and ultimate leaf fall due to excessive humidity. Excessive humidity not only causes an abnormal growth of foliage with hardly any cuticle and induces leaf fall, but makes the citrus plants very susceptible to sun scorch and consequent wilting and death on exposure to sun. In contrast, a plant kept under normal conditions is highly resistant to sun scorch.

A third seedling of *Citrus medica* of the same vigor and age was put in the moist chamber for the same period. This seedling again showed abnormal growth of foliage and leaf fall. The leaves were large and thin and developed almost no cuticle. This confirms the observations made previously. But this time the seedling, after its confinement in the moist chamber, was gradually exposed to the sun by first keeping it in shade for a week; it was then transferred to the sun after being well watered. The seedling stood the sun very well and showed no signs of scorching and wilting. This suggests that scorching and wilting in the first case could have easily been averted had the plant *A* been gradually brought to the normal outside conditions in order to allow the cuticle to develop properly and to train the stomata to respond to the changes of humidity in the air.

SUMMARY

1. A moist chamber, in which a seedling could be kept under excessively humid conditions well supplied with pure and fresh air, was designed.

2. In the excessively humid atmosphere of the moist chamber, there was practically no transpiration from the leaves, and at the same time there was a consequent increased growth of foliage. The new leaves were thin and had hardly any cuticle.

3. When the plant was taken out of the chamber and kept in the sun, white dried-up patches appeared on the leaf surface within four hours and ultimately the plant dried up, whereas the plant kept under normal conditions remained unaffected.

4. Scorching, wilting, and ultimate death of the plant could have been averted had the plant been gradually brought to the outside conditions so as to allow cuticle to develop and to train the stomata to respond to changes in the humidity of the air.

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DISTRIBUTION AND REPRODUCTION OF CANADA THISTLE IN IOWA

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(Received for publication February 20, 1933)

Canada thistle (*Cirsium arvense* Tourn.) is the cause of increasing agricultural loss wherever it has become established throughout its range. Its reputation generally precedes its advent, as evidenced by precautionary state weed laws. Much has been written concerning its control; nevertheless this plant is rapidly extending its boundaries. In the problem of control of Canada thistle in Iowa, questions pertaining to its distribution, reproduction, and growth habits have arisen which are not adequately answered in literature. For this reason, a study of the growth, range, sexual and vegetative reproduction of the plant, and viability of seed for the environmental conditions of Iowa was pursued during 1930, 1931, and 1932. The distribution of Canada thistle changes continually because of the natural establishment of new areas and on account of the work of eradication in progress throughout the state. Records are therefore subject to corresponding modifications. It is not the purpose of this paper to discuss control.

DISTRIBUTION OF CANADA THISTLE IN IOWA

General geographic distribution. The complete knowledge of the distribution of an introduced plant offers some basis for a prediction of its spread when introduced into similar climates. *Cirsium arvense*, the field thistle of Europe, is recognized by Hegi (1929) as indigenous throughout Europe, Asia, and Northern Africa (Tripoli). In Europe, it extends to Scandinavia 68° 50'N and northwest to Asia, where it has spread over Siberia, through China, Japan, and southeasterly to Afghanistan. Lansdell (1927) reports it in Natal, Transvaal, and the eastern Cape Province as a naturalized plant. Ewart (1930) states that it became naturalized in the province Victoria about 1887. Hansen (1922) infers that it was introduced into Canada at an early date through impure farm seeds. Detmers (1927) mentions that it was well known in Vermont as early as 1795, when it was officially recognized by law.

Climatic limit to range. In view of the fact that Canada thistle is established in Tripoli and Afghanistan in a latitude of 30°, and in Transvaal, latitude 25°, and since general climatic conditions of these countries are similar to those of the same latitudes in America, Canada thistle may be expected to establish itself farther south in the United States than its present southern limit of 37°N. *Cirsium arvense* in America is at present a common weed in the corn belt. In Russia it is a noxious weed of the cotton-growing districts

of Transcaucasia and Turkestan, according to Malvez (1931), who states also that it germinates best at high temperatures (25° – 30° C.). Kolokolnikov (1931) states that in the Vyatka region of Siberia, *Cirsium arvense* does not reach a normal development and does not flower at 58° – 59° N, but at 57° – 58° N it thrives.

Introduction and dispersal. Canada thistle is introduced into a region by various carriers and often at widely separated points. Isolated colonies which grow from a single initial seed or cutting consist of either staminate or pistillate plants; seeds are produced only after neighboring plants of both forms have been introduced, which may require several seasons. By local dispersal of seeds and by vegetative growth, colonies are rapidly extended over large areas.

Man as an agent. Of the various agents, by which Canada thistle is introduced, man is foremost. In a summary of seed analyses in Iowa from 1921 to 1924, King (1925) states: "The past year there has been a marked increase of Canada thistle in clover seed. This thistle has been common in states east and north of Iowa for a long time. It has been introduced by seed into many places in Iowa, but as yet one form, either staminate or pistillate, is usually found in these localities so that it does not seed freely. Canada thistle is not so likely to be in home-grown seed as in that from outside sources. It is comparatively frequent in alfalfa seed, which denotes an outside source. The same is also indicated by its frequency in alsike clover and white clover seed." In Iowa it is common along railroad tracks and highways. In a survey made in 1930, 1931, and 1932 it was located around beet sugar factories, greenhouses, cement plants, grain elevators, railway stations, and stockyards. In some of these locations it comes in straw used for packing or bedding. In the town of Colo it is common in gardens. When established in such places it spreads into fields. Specific instances of spread of Canada thistle by these related agencies will be discussed later.

Birds as carriers. There is a widespread belief among farmers that ducks and other waterfowls are the agents of distribution of Canada thistle "seeds." While a survey of biological records, including those compiled by Ridley (1930), does not indicate that Canada thistle "seeds" have been found in the excreta of waterfowls, it is generally recognized that these birds feed largely on herbage, fruits, and seeds. Finches, especially buntings, feed upon the akenes of thistles, though they are accredited with the destruction of these fruits by means of their bills.

McAtee (1915) found six "seeds" of thistle of unknown species in the gizzard of a common Mallard duck and two thistle "seeds" in the gizzard of the Black duck. He examined 45 ducks. Seeds of Compositae found were few. Marrot (1920) states that the Blue-winged teal feeds not only on water plants but upon upland plants, including members of the Compositae.

Investigation of the intestines of ducks. Through the courtesy of Dr. E. W. Henderson and Professor R. L. Cochran, facilities of the laboratory

of the Poultry Husbandry Department of Iowa State College were available. A variety of weed seeds including Canada thistle were fed to four Mallard ducks which had been confined 10 days until they were accustomed to their environment and able to feed normally. The seeds were mixed with a wet mash which constituted their regular food. The droppings during a period of 12 hours were examined, but no whole seeds were found. Three feedings were made. After each feeding fragments of food were removed and the floor of the cage was covered with fresh paper. Dissection of the alimentary canals of these birds showed that akenes stored at first in the crops were ground up in the gizzard. No whole seeds were found in the intestine.

In November, 1932, the intestines of 14 ducks, including a Green-winged teal, a Blue-winged teal, a Ruddy duck, a Shoveller duck, and 10 Mallards, were examined. The material was secured from Mud and Goose Lakes in northern Iowa by Mr. Logan Bennett. The contents of the intestines were expressed and suspended in water, and the liquid was filtered off through paper. In the dry residues from the intestines of most of the ducks were found only fragments of seeds, akenes, or other vegetative material in some cases mixed with mud. In the intestines of a Green-winged teal, a Ruddy duck, and a Mallard was found in each case a single *Potamogeton* seed. Another Mallard contained one seed of *Polygonum lapathifolium*. No thistle akenes were present.

Distance of flight and special mechanism of thistle fruits. The plumed fruit is undoubtedly the chief source not only of local dispersal but of introduction at points distant from its source. Numerous observations are recorded in literature on the facilities for dissemination of the plumed fruit of Canada thistle. Small (1918) gives an elaborate account of the mechanics of the flight of plumed akenes. He states that in the case of the dandelion fruit as long as the relative humidity of the air remains above 77 per cent and the fruit does not encounter any obstacle, a horizontal wind at 1.97 miles an hour can carry it to any distance. These data show that the plumed fruit is well fitted for migration on air for an indefinite distance. The almost constant winds of Iowa with a much higher velocity than the rate cited in the experiment magnify the facility of travel. Failure to destroy initial patches of introduced thistles, combined with natural mechanisms for flight of the fruit, is accountable for unhampered spread of the thistle.

REPRODUCTION OF CANADA THISTLE IN IOWA

Seed production

The belief is general that Canada thistle does not produce seed in central and southern Iowa. The following records give some concept of the advance made by Canada thistle in Iowa during a period of 40 years, during which period there is evidence of seed production.

Early records. The first weed law of Iowa, codified by the Sixteenth General Assembly, reads as follows: "Be it enacted by the General Assembly of the state of Iowa, that if any resident owner of any land in this state after having been notified in writing of the presence of Canada thistles on his or her premises, shall permit them or any part of the root to blossom or mature, he or she shall be liable to a fine of five dollars and cost of collection for each offense." (April 7, 1868.)

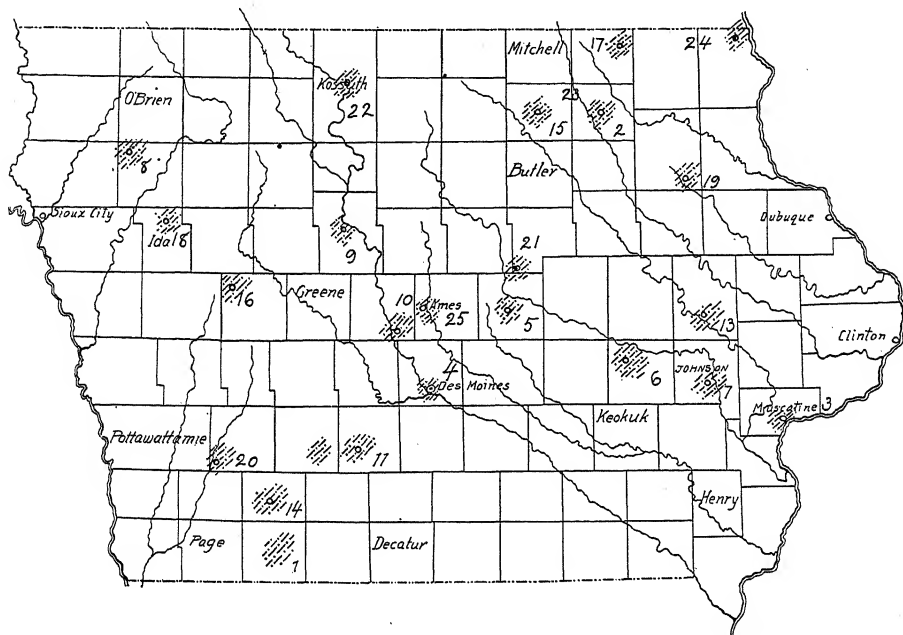


Fig. 1. Diagram showing the distribution of Canada thistle in Iowa in 1898: (1) Taylor Co., (2) Chickasaw Co., (3) Muscatine, (4) Des Moines, (5) Marshalltown, (6) Iowa City, (7) Johnson Co., (8) Marcus, (9) Ft. Dodge, (10) Randall, (11) Winterset, (12) Ladora, (13) Cedar Rapids, (14) Corning, (15) Charles City, (16) Maple River, (17) Cresco, (18) Sac City, (19) Oelwein, (20) Griswold, (21) Conrad Grove, (22) Bancroft, (23) Lawler, (24) New Albin, (25) Ames. (After Pammel, 1898.)

With reference to seeding in Iowa, Pammel (1898) states: "I have repeatedly examined the thistle in Wisconsin, Iowa, Illinois, and Missouri, and with few exceptions seeds have not been found. Canada thistle occurs in great quantities in and about Chicago, especially west and north. The weed is common in and about Milwaukee, and there I found seed in abundance." A map from this paper (1898) showing the distribution of Canada thistle in Iowa records twenty-four locations in as many counties (fig. 1). One of these patches occurred in Taylor county in the southernmost tier of counties. Pammel (1891) writes of receiving from Chickasaw and Taylor counties specimens of Canada thistle which had a good many flowers but no seed.

He says: "In these places as well as many other parts of the West it spreads almost entirely by its underground stems, producing few 'seeds.' It will not do, however, to lay too much stress on the fact that it is not likely to become troublesome in the West since it does not 'seed.'"

Unpublished data secured by Pammel for 27 counties and Hayden for 31 counties in 1925-1928 in connection with a weed survey of Iowa, and records of Pammel and King (1926) in 50 counties, supplemented by recent statements (1933) of county agents from 65 counties, show that Canada thistle has spread to every county in the state (fig. 2). An increase in the area oc-

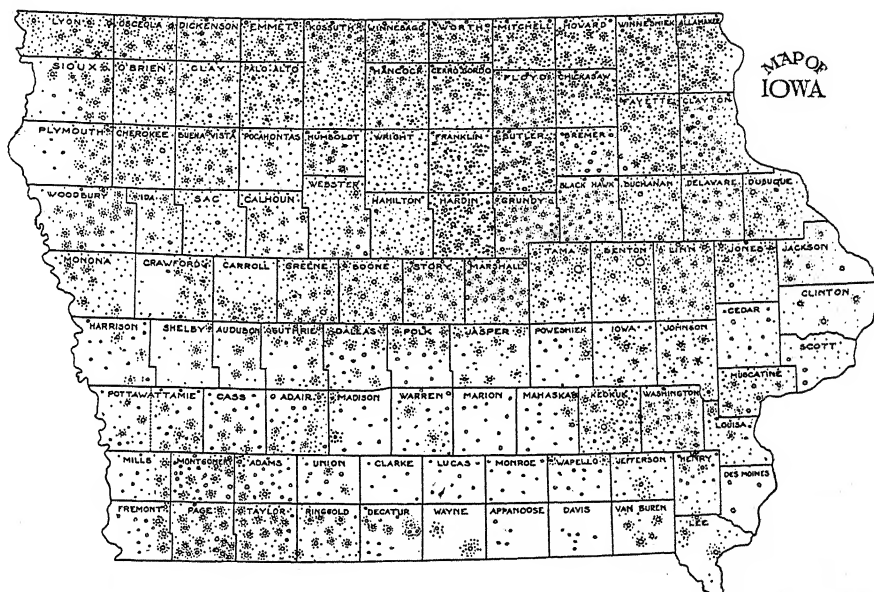


Fig. 2. Diagram showing the approximate distribution of Canada thistle in Iowa. The relative presence of the plant is shown in terms of dots representing occasional plants; and circles representing patches of one or more acres. The approximate distribution is diagrammed to locate districts where Canada thistle is known to be infrequent, frequent, or abundant.

cupied in 1926 is commonly reported. Iowa has long maintained preventive measures against Canada thistle, a fact which is shown by the date of the first weed law (1868). This work is at present continued under the leadership of Porter (1931) in cooperative projects with farm bureaus.

Recent records. Efforts were made in the summer of 1930 to determine the status of seed production in central and southern Iowa. The theory has been advanced that climatic differences account for the alleged absence or sparseness of seed production in southern Iowa. The harvesting of crops, which may be taken as an indicator of season, occurs two to three weeks earlier in southern than in northern Iowa. Essentially the same crops are

grown in both places, though strains and varieties are selected with reference to the time required for maturity. The distance between the north and south borders of the state is 200 miles. Observations have been made on seeding of the thistle in Cerro Gordo, Mitchell, Hancock, and Worth Counties in northern Iowa; in Story and Boone Counties in central Iowa; and in Page and Pottawattamie Counties in southwestern Iowa.

Northern Iowa. In Cerro Gordo County six miles northwest of Mason City some large patches of Canada thistle were found in woodland areas and on adjoining farms. One area of about two acres over which thistles were scattered contained a number of small patches 12 or 15 feet in diameter. Ten patches were examined, and in all but one the plants were found to be exclusively staminate or pistillate (fig. 3, 4); seed was abundant, every flower (as



Fig. 3 (left). Seedless heads of staminate-flowered plants. Fig. 4 (right). Heads of pistillate-flowered plants bearing akenes containing seeds ready to set sail July 22 in northern Iowa.

many as 70-75) in the mature heads commonly producing a seed. Some of the plants were in full bloom, though many bore mature fruits which were floating off in every gust of wind (July 22). Some of these floating fruits lodged in near-by brush piles; others fell at the edge of the woodland clearing; others settled in an orchard across the highway, where a year after young

plants were found to have been established. Each of six or more brush piles of this clearing harbored a tangle of thistles. A woodland clearing on the opposite side of the road about a quarter of a mile distant contained several colonies of blooming and fruiting thistles. In colonies of pistillate plants in the first woodland clearing, where the staminate plants were 25–100 feet distant, considerable seed was found—20 or 30 seeds to a head. In colonies 500–600 feet apart, few seeds were noted—only 2 or 3 in a head. In the woodland clearing across the road, in an area of about an acre, were two staminate colonies 25–50 feet in diameter, each about 25 feet from a pistillate patch. Here abundant seed was found in the pistillate heads—50 to 100 seeds to a head. The conditions found in these areas indicate that wherever the staminate or pistillate plants are adjacent mature seed will be produced. Similar observations were made concerning seed production in Winnebago County, in pastures north of Forest City, and in fields four miles south of Emmons, Minnesota. In Cerro Gordo, Mitchell, Hancock, and Worth Counties, as many as 20 patches were examined in which were only staminate or pistillate plants, indicating the probable origin of each patch from a single seed. These patches were located in pastures, by roadsides, or on railroad right-of-ways. In Mitchell County a hundred-acre pasture woodlot bore numerous seed-producing thistle colonies. The akenes were mature on the twentieth of July, which was nine days before the date on which supervisors required them



Fig. 5. Cutting thistles after the seed has ripened.

to be cut (fig. 5) in 1929. The time for cutting has now been changed to an earlier date; but the advances made by the thistles before this change cannot be counterbalanced in a single year.

Central Iowa. In Story County a survey made in 1932 shows that in each

township in the county thistles are present in centers of distribution of 1-40 acres. Mr. H. P. Hanson, when County Agent in Story County (1930), stated that nearly every farm had a patch of thistles. That the plant has increased comparatively rapidly in the past 10 years appears evident, since previous to the last decade farmers appeared to be unfamiliar with the plant, but now it is widely recognized. Some detailed observations made with reference to reproduction occurring in these areas are shown below.

Notes made on August 23, 1930, in Franklin Township between Ames and Story City, revealed 25 patches in 20 per cent of which seed was found. In all cases where staminate and pistillate plants were found in the same colony or where they grew within 20 to 200 feet of each other, seed was produced.

Four miles north of Ames, Iowa, Canada thistles in 1930 were growing as hedges along fence rows on both sides of a lane along which cattle passed. On a farm across the highway a hedge along a cattle driveway stood uncut after seeding time. In a pasture near-by numerous colonies had started, and in neighboring cornfields and pastures for several miles on either side were patches of thistles. In the adjacent cultivated land plants were being broken into fragments by the plow, giving rise to many new colonies. From the eroded banks of a stream running through this pasture fragments of Canada thistle roots were hanging and portions of the plants lay in this ditch where they could be carried away by floods to a near-by county ditch tributary to Skunk River. Newly established thistle colonies occurred at intervals farther down the ditch in the cornfield. The instance cited just above illustrates how thoroughly a region may be seeded down to Canada thistle before farmers are aware of the condition.

The initial colony in this pasture, according to the resident on this farm, had been established for 12 years. The hedges on the two adjoining farms were not cut before seeding time in the summers of 1931 and 1932, though the initial patch in the middle of the pasture was cut. The thistles in the hedges continued to grow, produce seed, and spread without interference.

Three miles east of Ames a field of 40 acres of oats was cut and burned in the summer of 1932 because the thistles outnumbered the oats. The thistles were reported seeding when cut. The field was fallowed for the remainder of the summer, but the cultivation was done at such times that the thistles made a copious growth of leafy shoots. The cutting of vegetative fragments by the plow has undoubtedly assisted in spreading the plant over the field. Much of the area bears as many as 150 shoots to the square yard, as determined by 100 quadrat counts. These thistles, according to community records, have been established for 25 years. Small patches were located on surrounding farms. This illustrates how well-established areas can extend the distribution of the plant.

In Boone County many isolated patches were observed. One patch about eight acres in area was located in a cornfield. No seed was found in the areas noted, nor in any case were both staminate and pistillate plants present.

Most of the patches located were cut at this time (August). Mr. H. E. Schroeder, County Agent of Boone County, stated, however, that nearly every farm had in 1930 a patch of thistles.

Southern Iowa. Limited observations were made in southwest Iowa during the first part of August, 1930. Two cut-over areas were observed in pastures in Page County. The plants were pistillate only; no seed was found. One large patch in Page County three miles south of Shenandoah, with both staminate and pistillate plants, bore seed. Near Shenandoah on and near nursery property several established thistle patches were noted by Mr. Elbert O. Brown. Some of these grew along a high creek bank for a distance of 200-300 feet on each side and had spread into a pasture. It is reported from this community by a weed commissioner that a seed dealer dumped a quantity of seed refuse into a creek on one of his experimental plots. Other patches in this county were growing on creek banks. One large area of several acres was receiving fallow treatment. Six isolated patches seen in Pottawattamie County were either staminate or pistillate. No seeds were produced.

The rapid increase of thistles in southern Iowa suggests that they are spreading by seed throughout the state. Effectiveness of wind dissemination of the "seed" makes the control of Canada thistle more difficult than that of weeds which do not have facilities for such distribution. Spread of thistles in agricultural seed is a slow process, likewise their spread for any distance by vegetative cuttings as by the cultivator; but wind-borne fruits from a good-sized patch of thistles can in two or three seasons infest a township. This is evident from the distribution in Story County, where in nearly every township a well-established seed-bearing patch may be found around which the adjoining farms bear small but vegetatively enlarging colonies. One such colony by a roadside is known to have extended its border 20 linear feet in a single season. Rogers (1909) reports from Colorado extension of 40 feet in a season.

Comparison of early records and recent records. Reference to a distribution map of Canada thistle in Iowa made by Pammel in 1903 shows only one entry of thistle plants in the southernmost tier of counties. This record was in Taylor County. A map published by Pammel and King (1926) shows entries in Mills, Page, Taylor, Ringgold, and Wayne Counties. Pammel (unpublished data, 1926) reported Canada thistle as not infrequent in cultivated fields of Fremont County. Some estimates obtained from county agricultural agents in this southern tier of counties in 1931 show distribution as follows:

In Page County, according to Don T. Griswold, there are 21 marked locations rather uniformly distributed. In Taylor County it is estimated by T. H. Isaac that 50 per cent of the farms have each 10 to 20 acres of thistles. Though 40 years have elapsed since Pammel (1891) first recorded receiving seedless specimens from Taylor County, in 1930 this county leads in reported distribution of Canada thistle, and the plant is now well established in the

whole of the lower tier of counties. Ringgold County has Canada thistles in every township. In Wayne County, states W. J. Roudabush, many localities have Canada thistle. Though Appanoose County is relatively free, according to Leo Bowdish four areas in the northwest quarter of the county are known. Four localities are recorded for Van Buren County by Arthur J. Secor, and several localities are noted in Davis County by Walter Brown, and in Lee County three widely separated patches which are said by W. P. Calvert to spread very rapidly. More thistles are recorded for the western half than for the eastern half of the lower tier of counties.

The steady spread of Canada thistle and the increase in the size of the colonies indicate increased numbers of patches where staminate and pistillate flowering plants are growing in close proximity. The economic insecurity of agricultural pursuits from 1921 to 1932 which resulted in relaxed vigilance in farming operations has favored the occupation of new territory by Canada thistle.

Floral structure in relation to seed production. The flowers of Canada thistle are arranged, on a common stem axis, in clusters of approximately 100. Such a flower cluster (fig. 6) is known as a head. The flowers vary in color

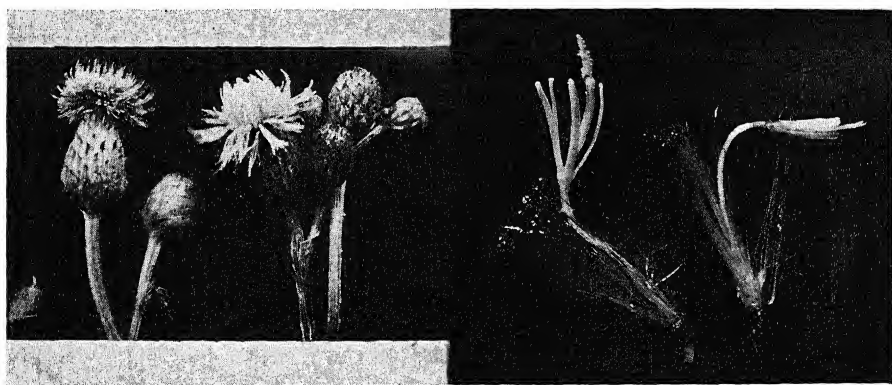


Fig. 6 (left). The elongated pistillate head (left) and the broad staminate head (right). Fig. 7 (right). Staminate flowers (left) have corollas with long lobes. Pistillate (right) flowers have corollas with short lobes.

from purplish rose or pale pink to white. Though all the flowers are perfect, each bearing stamens and a pistil, they are functionally either staminate (pollen-bearing) or pistillate (seed-bearing) (fig. 7). The pistils of staminate flowers are abortive and the stamens of the pistillate are abortive. The abortive stamens are deep purple in color, but the normal stamens are colored like the corolla, usually pink.

Detmers (1927) amply reviews the diverse statements of many writers regarding the structure of the flowers. She shows experimentally that plants grown from seed to maturity are either staminate or pistillate, and therefore

dioecious. Some plants bear staminate heads only, others bear pistillate heads only (fig. 6). Isolated pistillate colonies bear no seed, but mixed colonies including both staminate and pistillate plants bear abundant seed. Pistillate colonies growing within 200–300 feet of staminate colonies, a distance which makes insect pollination possible, bore some seed. A count was made of akenes per head of plants growing in various localities in northern Iowa. A tabulation of these data follows.

TABLE I. *The range of akene production per head*

Location	Date	No. of heads	Number of akenes		
			Max.	Min.	Ave.
Hamilton Farm, Hawarden, Iowa	July 18	27	80	16	52.85
½ mi. south of Emmons, Minn., Winnebago Co.	" 29	33	104	15	66.06
" " " " " " "	" "	16	80	18	49.06
" " " " " " "	" "	18	83	25	55.3
6 N.W. Mason City, Cerro Gordo Co.	" "	16	56	11	41.93
" " " " " " "	" "	24	67	6	28.7
" " " " " " "	" "	13	79	15	45.84
" " " " " " "	" "	17	8	1	5.0
" " " " " " "	" "	20	87	17	38.95
Story County	Aug. 2	23	38	14	24.13
" "	" "	20	57	15	28.5
" "	" "	29	68	12	37.1

Each head bears approximately one hundred flowers. The number of seeds per head, excepting the White Thistle, averaged about 46. In these counts about 50 per cent of the flowers in the head bore seed. However, as many as 98 seeds have been counted in one head.

Viability of seed. Twenty samples of 100 seeds each, of six-months-old seed tested on blotters in a germinator at about 87°F., ranged in viability from 10–27 per cent. The two-year-old seeds from the same sample tested in sterilized soil in August in the greenhouse had a viability of 15–43 per cent. Ten samples of 100 seeds each of two-year-old seeds were tested in sterilized soil in February with a viability of 38–71 per cent and an average of 50 per cent. Freshly gathered Canada thistle seeds collected in August in Hamilton County, Iowa, averaged as high as 95 per cent in germination. The seeds germinated were gathered in a variety of locations in Cerro Gordo, Winnebago, and Story Counties. The geographic location appeared to have no effect on the viability of the seeds.

The akenes were gathered from various heads and the smaller seeds sifted out. Since the flowers in the middle of a head mature later than those at the edge, some of the seeds from a head are probably less nearly mature than others and might not germinate as readily as if left in the field until matured. Hence under the most favorable conditions, the viability of Canada thistle "seed" in nature is probably higher than the laboratory experiment indicates. Since by means of only one successful seed the thistle may spread over an area of several acres, the most important consideration is not how large a

percentage of seeds will germinate, but whether or not any germinable seeds are produced. In counties where thistle patches are isolated, there may sometimes be found areas of 1-10 acres consisting of staminate or of pistillate plants only, but when the two types of plants occur within a few hundred feet, so that pollination may take place through insect visits, "seeds" appear in quantity. As the distance between staminate and pistillate patches increases, fewer "seeds" are found, and where the patches are isolated no perfect "seeds" are found in the pistillate flowers. The akenes appear thin and shrivelled.

Time of germination. The seeds of Canada thistle will germinate immediately after seed is mature in July or August, and are able to produce, by the winter season, rosettes which have a root system sufficiently established that they may bloom the next season. Seeds which do not germinate in the fall may germinate in the spring. These plants bloom in the second season. Seeds lying in the ground or in flats in the greenhouse do not all germinate at once but at successive intervals. This delayed germination produces a succession of plants in different stages of development. In Story County, the spring germination was noted in the field in April and May.

The Seedling

Canada thistle seedlings emerge from the earth by the elongation of the hypocotyl. They bear two cotyledons which are thrust from the earth with appressed faces and usually in an upright position. The cotyledons are at first completely enclosed, but soon emerge from the shell of the akene which rests for a time on the tips of the cotyledons before they unfold and drop the akene shell and seed coats. Sometimes the growing hypocotyl becomes arched, pulling the cotyledons out after it. The cotyledons are oval to ovate in shape and with somewhat frosty-puberulent surface. The seedlings are not readily distinguished from those of many other members of the sunflower family until the foliage leaves appear.

The first foliage leaves are ovate to round in shape bearing regularly spaced, long, coarse, marginal hairs which soon become spinose as the margin of the leaf becomes serrate and then lobed.

The developing slender tap-fibrous root system varies considerably. A seedling bearing two foliage leaves may have a simple, branching root system from 1-6 inches long (fig. 8). The main root soon thickens, and as food manufactured by the leaves accumulates in this root, lateral shoots on the root are produced which may run obliquely, horizontally, or arching upward through the earth (fig. 9, 12). The main vertical root or branches of the root produce two kinds of structures:

Roots, which grow horizontally from the main or vertical roots. These sometimes arch downward or upward. This arching character is mentioned by Lund and Rostrup (1901) and by Rogers (1927). These roots ramify eventually into a complex interlaced system producing roots only, deep in the

earth (fig. 12). When cut into sections, the roots develop stems or roots at any point (fig. 11).

Stems may originate (1) from lateral buds on the original vertical roots usually within the first foot below the soil and grow obliquely upward to the surface, or (2) from buds on the arched ascending or descending branches

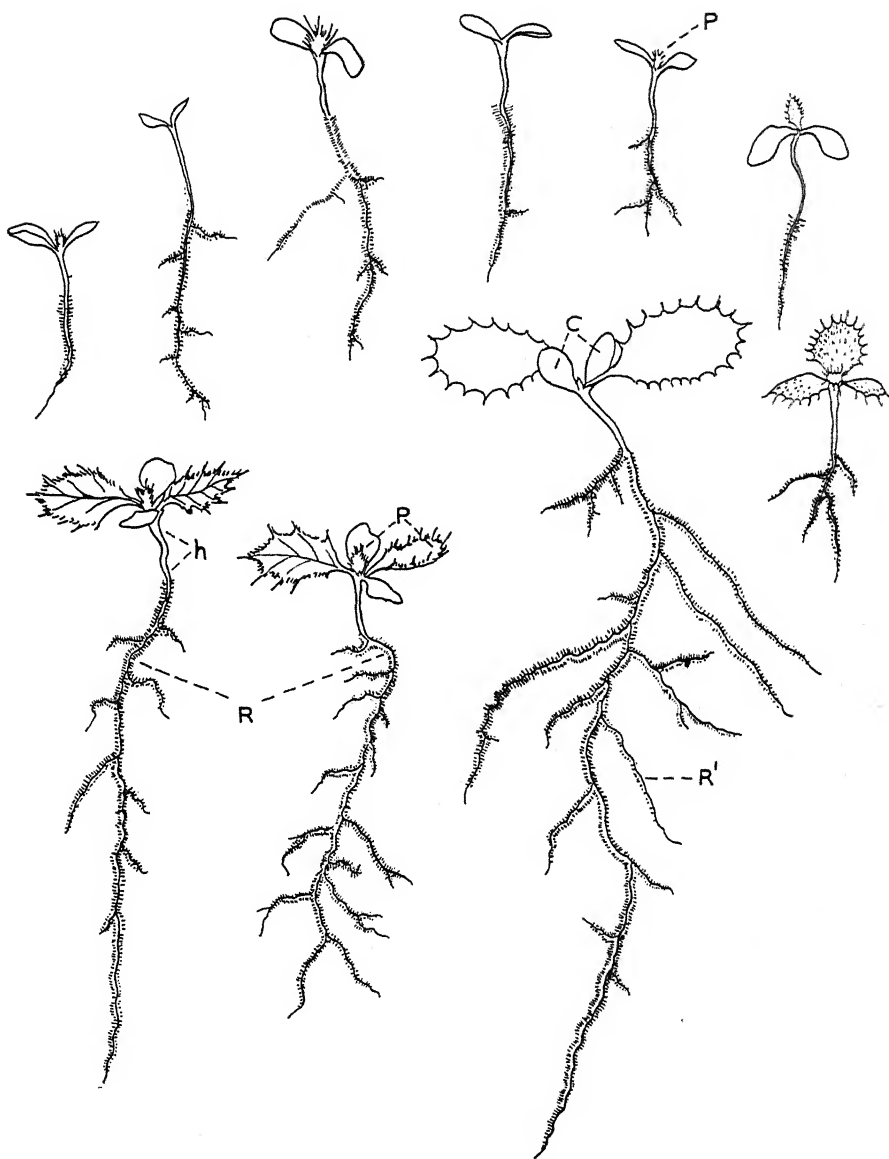


Fig. 8. Seedlings showing various aspects of the primary root system (*R*); cotyledons (*c*); plumule with first foliage leaves (*p*); hypocotyl (*h*).

of roots, emerge and expand at the surface of the earth into leaf rosettes which augment the food supply and enable the plant to repeat again and again this procedure of extending its domain. These root-borne shoots may

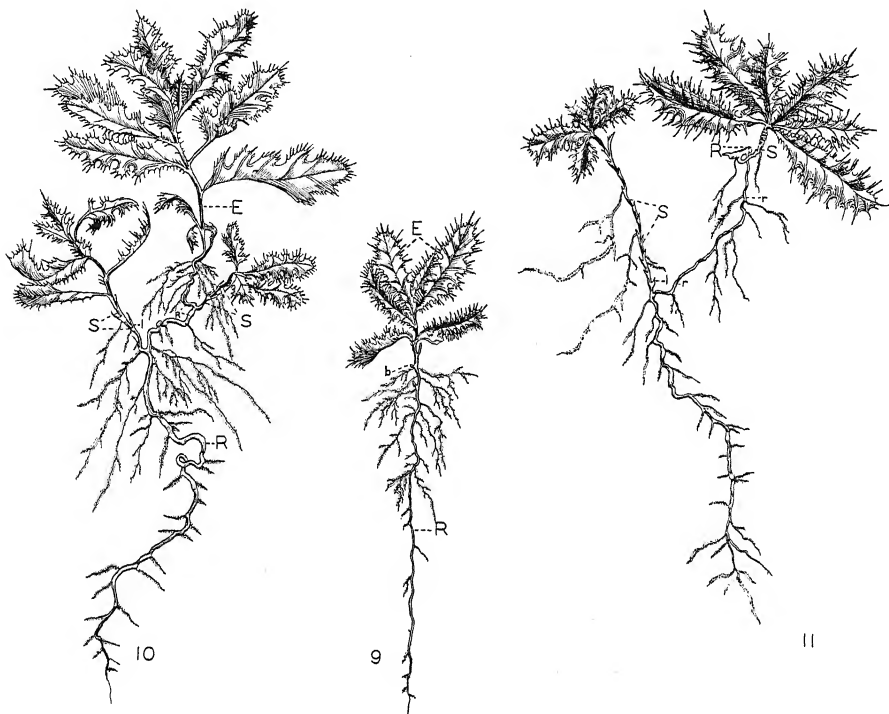


Fig. 9-11. Fig. 9 (center). Six-months-old seedling, grown in greenhouse bearing root buds (*b*), which later develop into stem shoots (*S*) on the older plant. Fig. 10 (left). Primary root (*R*) bearing stem shoots on ascending rhizomes (*S*); epicotyl (*E*). Fig. 11 (right). Regeneration of a Canada thistle plant six months old grown from a root cutting (*R*) one-half inch long and one-eighth inch in diameter; new roots (*r*); stem or ascending rhizome (*S*); leaf-scales at nodes of stem (*l*).

be regarded as rhizomes (fig. 9, 12), since their histological structure is that of a stem. They bear roots, buds, and leaves at the nodes and are uniformly thickened throughout their length.

The rootstock or rhizome of Canada thistle

In a broad sense a rhizome or rootstock may be regarded as a somewhat uniformly thickened underground stem whose direction may be horizontal, vertically ascending, or descending. All of these conditions are fulfilled by the subterranean system of *Equisetum arvense* (Horse-tail), whose underground stems are essentially rootstocks having nodes bearing conspicuous leaf-scales, buds, and fibrous roots and whose histological structure is typical of

stems. Brenchley (1920) recognizes such a concept of rootstock, though in illustrations she calls the horizontal branches of the underground system, stems. Most writers refer to prostrate rootstocks only. In Canada thistle the rhizomes occur as vertical or obliquely ascending shoots, growing from scattered buds on the side of vertical or horizontal roots.

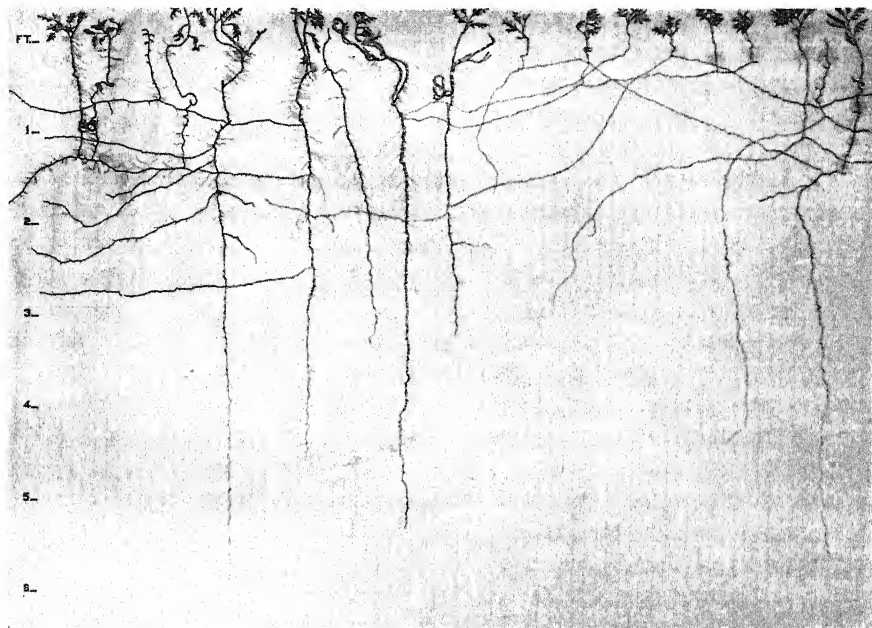


Fig. 12. The subterranean system of a single Canadian thistle plant showing the vertical absorbing and storage roots and the horizontal or arching roots bearing upright stem shoots.

Origin. Rootstocks appear to have several sources of origin: (1) from the epicotyl of the seedling as in *Agropyron repens*, (2) from the hypocotyl of the seedlings as in *Convolvulus arvensis*, as shown by Beijerinck (1887), and (3) from the root as in Canada thistle (fig. 12).

The subterranean system

Statements regarding the structure of the subterranean system of Canada thistle are various. Microscopic examination of the underground parts supports the investigations of Pammel and Fogel (1909) with drawings by King that Canada thistle is propagated by a root bearing adventitious buds. Several excavations were made in cornfields for the purpose of studying the subterranean system of the Canada thistle. The technique employed was as follows: A trench about 6 feet wide, 10 feet long, and 8 feet deep was dug

in a patch of thistles. After a typical vertical section of the root system was exposed, the earth was removed carefully by the aid of chisels and icepicks, and drawings of the general aspect and sections of the detail of the roots were made in the trench. The roots were then removed from an area represented by a block of soil about one foot in transverse measurement, 7 feet deep, and 10 feet in length (approximately in one plane), and were taken to the laboratory, where the system was reconstructed upon a large sheet of cross-section paper, on which the roots were laid. By the aid of the drawing made in the trench and the carefully excavated roots, a detailed drawing of natural size was made. Figure 12 shows a section of a root system growing in loam soil, the upper six inches of which had been cultivated early in the season, but which during a period of drought had become like hardpan. Some of the shoots which broke through this crust assumed a coiled aspect. For about 5 feet below the crust the soil was friable and abundantly penetrated with roots extending both vertically and horizontally, with numerous upright stems emerging from the horizontal roots. At depths of 1 to 3 feet horizontal roots grew out from the vertical roots. These at length arched upward toward the surface of the soil extending the margin of the colony. The young roots usually terminated in several fine branches bearing the root hairs. The horizontally running food-storing roots bear few secondary branches, but the vertical shoots (rhizomes) bear numerous fine fibrous roots, which appear to facilitate absorption in surface and sub-surface soils, where the root system is frequently cut by the cultivator. Cultivation stimulates the growth of horizontal roots, thereby increasing the number of new upright shoots borne by the horizontal runners. The vertically descending roots terminated in clay at the water table at a depth of 6 to 7 feet. However, various depths of penetration have been recorded. Rogers (1928) reports a depth of 9 feet in Iowa and Colorado; Lund and Rostrup (1901) record depths of 8 feet in Sweden, and Malvez (1931) states that Canada thistle roots penetrate to a depth of 18 feet in the black earth of the cotton regions of Russia. It is our observation that the length the roots ultimately attained seemed to be determined by the depth of the water table, for the terminal roots penetrated to saturated soil. Horizontally running roots appeared to connect the entire patch, as was shown by surface excavations in the upper two feet. The horizontal roots bore relatively few secondary roots, but numerous buds. The shoots which sprang from these buds answer the description of vertical root-stocks, for they are uniformly thickened and bear buds, leaf-scales, and roots at their nodes. The buds on the nodes occur at regular intervals and fibrous roots occur either at nodes or on internodes.

Texture of the soil and depth of the water table modify the development of the underground system of Canada thistle, so that various and quite different representations of its habit have been found. This condition would at least suggest that, though the root system has a fairly typical topography, it is quite flexible in its habit, dependent on the conditions of its immediate

environment, such as soil texture, fertility, and moisture, as well as the vegetative cover. Lund and Rostrup (1901) illustrated several growth patterns of root systems excavated from soils of Sweden.

Means of vegetative increase

Vegetative increase may occur in two ways: (1) The upright shoots or rhizomes are able to produce roots and buds at any node, hence when cut in sections new plants may grow from the nodes; (2) the roots, both horizontal and upright, may produce buds and roots at any point, which gives them ability to grow more new plants per unit of mass than the stem shoots, which have buds at intervals of one to two inches.

Roots both horizontal and vertical, when cut into sections $\frac{1}{8}$ to $\frac{1}{4}$ inch in length, were able to produce shoots which grew into plants. Longer pieces containing a greater quantity of stored food produced larger plants in less time than did the smaller pieces. If a portion of stem bears several nodes, usually only one bud will grow; but if the stem be cut leaving one node to a cutting, each bud will develop. One hundred pieces of young root $\frac{1}{2}$ inch long and $\frac{1}{8}$ to $\frac{1}{4}$ inch in diameter produced shoots 95 per cent, when grown under favorable conditions in the greenhouse in midsummer. The internodal lengths of cuttings vary, depending on the type of soil and vigor of growth, but they average a length of at least an inch. On this basis of inch-lengths for stem cuttings each bearing a bud, 2 to 4 times as many bud shoots can be made by root cuttings (which admit of smaller bud-producing divisions) as by stem cuttings. The number of active bud shoots developed depends, of course, on the amount of segmentation of these organs. The removal of the terminal buds from stem shoots increases the number of shoots produced by dormant lateral buds, there being as many potential buds as there are nodes, whereas the creeping and vertical roots may be cut into sections $\frac{1}{4}$ to $\frac{1}{2}$ of the length of the stem sections, and still produce shoots.

When cuttings are taken from stems about a year old, the stems are elastic in texture, and microchemical tests show that the cells are densely filled with starch, sugars, inulin, and fats. The tissues are light brown or whitish in color. Such cuttings germinate readily. When half-inch cuttings in lots of one hundred were made from underground stem shoots or roots at the end of the flowering season in August, 1930 and 1931, they showed 5 to 10 per cent of growth or none. At this time these organs had largely exhausted their food supplies, as shown by microscopic examination, and had become brittle in texture. The old, exhausted, brittle, vertical roots in a thistle colony become first hard and brown, and later soft and black as they disintegrate. The running roots in several seasons die at one end as they extend the network of new plants. There are always enough new ramifications of the running roots to keep the surface of the ground well populated with aerial shoots which arise chiefly from the horizontal running roots. When a patch

is cultivated and connection broken with the deep vertical roots, the tendency is for many surface horizontal portions to produce several stem shoots, and for the vertically descending roots to grow deep-running horizontal roots 1 to 3 feet below the surface (fig. 12). These horizontal roots then send up many vertical stem shoots or rhizomes from buds springing from any point on their surface. Though horizontal running roots may grow obliquely downward for several feet, they curve upward near the surface before sending up stem shoots. Most of the root-borne stem shoots occur in the upper foot of soil.

SUMMARY

Canada thistle (*Cirsium arvense* Tourn.) is able to produce seed throughout the climatic range of Iowa.

This seed has relatively high viability; under favorable conditions it may germinate without a rest period. Much variation in percentage of viability is manifested under differing conditions.

The presence of staminate and pistillate plants in the same vicinity, usually within two to three hundred feet, is necessary to insure pollination and therefore seed production.

The content of the intestines of 20 ducks which had been feeding on plant seeds and fruits revealed no significant evidence that weed seeds or fruits were disseminated by these fowls in their alimentary canals.

The plant may spread vegetatively by cuttings of the horizontal or vertical roots and by cuttings of the upright subterranean stem shoots which bear nodes. Portions of root or of stem $\frac{1}{8}$ to $\frac{1}{4}$ inch in diameter and $\frac{1}{2}$ inch long will produce new plants under favorable conditions.

Root cuttings are able to produce two to four times as many shoots as stem cuttings per unit length.

Either rhizome cuttings or root cuttings taken when their food supply is low or exhausted produce few or no bud shoots.

The subterranean system consists of vertically descending and horizontally spreading roots, both of which bear obliquely or vertically ascending stem shoots or rhizomes. The subterranean system has a fairly typical topography, though it is quite flexible in its habit, dependent on the conditions of its immediate environment.

Introduction of Canada thistle into new communities appears to be incident primarily to industrial activities of man. The local dispersal of the plant seems to be the result largely of the efficient mechanism of the plumed fruits, combined with widespread failure of human agency to destroy the plants before fruiting occurs.

In a period of 40 years, Canada thistle has increased from scattered initial spots to established areas in every county in Iowa.

Canada thistle may advance southward in the United States 7-10° beyond

its present range, according to the climatic range which it now occupies in European, African, and Asiatic countries.

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A FURTHER STUDY OF DISMAL SWAMP PEAT¹

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In an earlier paper (Lewis and Cocke, 1929) the history of the vegetation of a limited area of the Dismal Swamp near Wallaceeton was discussed on the basis of the method of analysis of fossil pollen. The present paper will be devoted to a similar study of another area of the same swamp extending from Suffolk along the Jericho Ditch to Lake Drummond (see map, p. 375). A study of the diatoms and sponge spicules is also included in this paper.

DISCUSSION OF THE PROBLEM

Historical

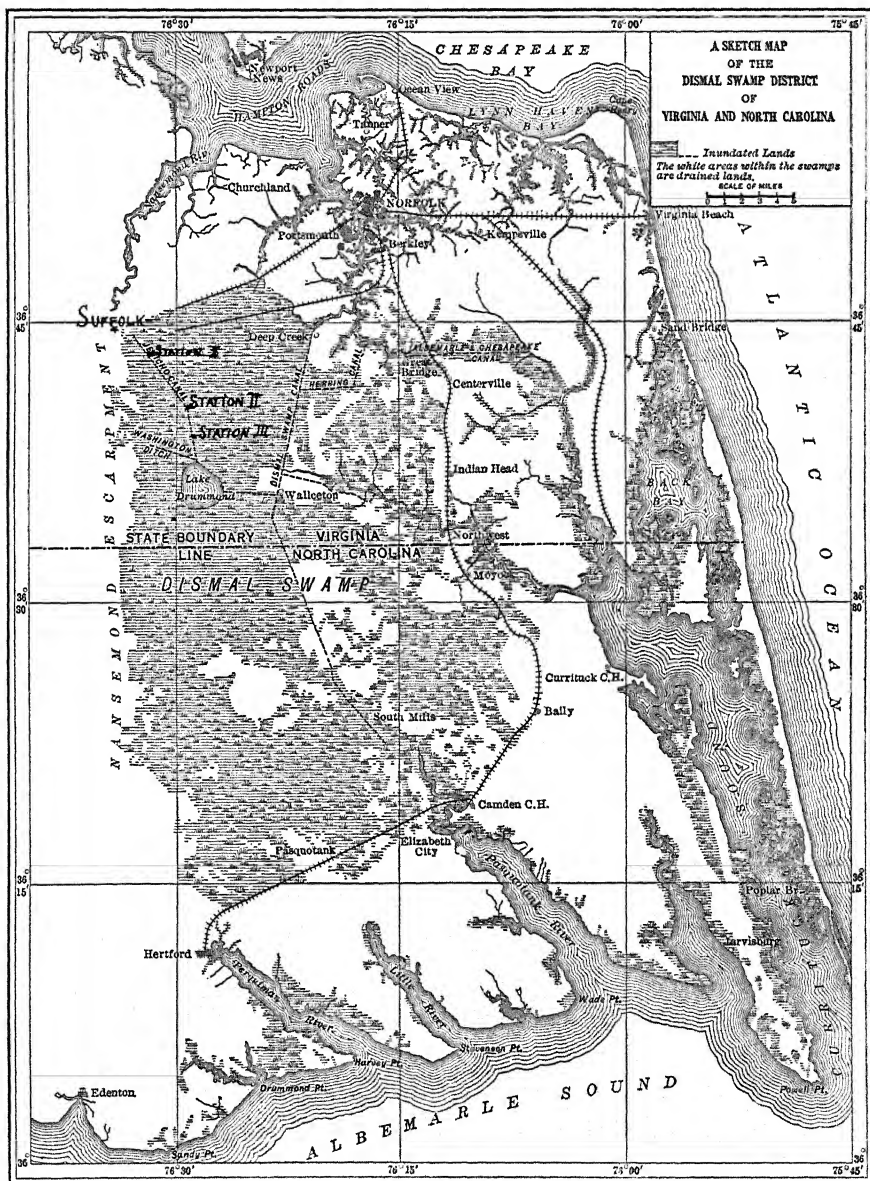
As early as 1895, Dr. C. W. Weber of Bremen realized the possibility of fossil pollen analysis as a means of studying succession of vegetation; he counted pollen grains and calculated the percentage of *Pinus* and *Picea* (Erdtman, 1924, p. 450). Extensive investigation in the study of peat bogs was begun by Dr. G. Lagerheim of Stockholm in the first decade of the present century. Von Post perfected the method and gave great impetus to the work. European scientists were quick to appreciate the possibility of studying bogs by this method and of correlating their findings with those of the archaeologists, who had already investigated the peat deposits extensively. Thus they entered the work enthusiastically, and as a result, important studies of many of the bogs of Central and Northern Europe and the British Isles have been made, and much valuable information concerning climatic change and plant succession of these regions has been obtained. Among the investigators in this field none has been more active than G. Erdtman.

Less interest has been manifested in the bogs of America, and until the publication of Väinö Auer's paper on some of the Canadian bogs in 1927, the peat deposits on this side of the Atlantic remained uninvestigated from the standpoint of pollen analysis. Since this time, however, much work has been done on American bogs. Sears has published several important papers on the glacial bogs of the Central United States. Lane, Draper, Sears and Couch, Voss, Wilson, and Potzger have also made contributions on the bogs of this area. Bowman has published an account of his studies on some Canadian peat deposits. Auer is continuing his work on the bogs of Canada also and has published several papers in this field. The work of Lewis and Cocke (1929) on the Dismal Swamp is so far the only contribution on non-

¹ Contribution from the Miller School of Biology, University of Virginia.

glacial swamps. In a recent paper Erdtman (1932) has a map showing the areas of North America which have been investigated as well as those which are being investigated by the method of fossil pollen analysis. A list of American workers in this field is also included in this paper.

The study of peat deposits by the method of fossil pollen analysis is based on certain fundamental principles: (1) The distribution of wind-blown pollen



throughout a vegetational area is more or less uniform; (2) the type of pollen is definite for each family and sometimes for genera and species; (3) the nature of the pollen wall is such that the grains and even the precise structural details are preserved indefinitely in peat. The first of these principles is essentially true. In applying the principle, it should be noted that some trees may produce much larger quantities of pollen than others. For instance, *Platanus* produces a small amount of pollen, while *Betula*, *Alnus*, *Pinus*, and others produce an abundance. Furthermore, some pollen will be scattered much more widely than others. Because of the peculiar structure of pine pollen and the height of the trees, the grains are blown for greater distances than the pollen of many trees.

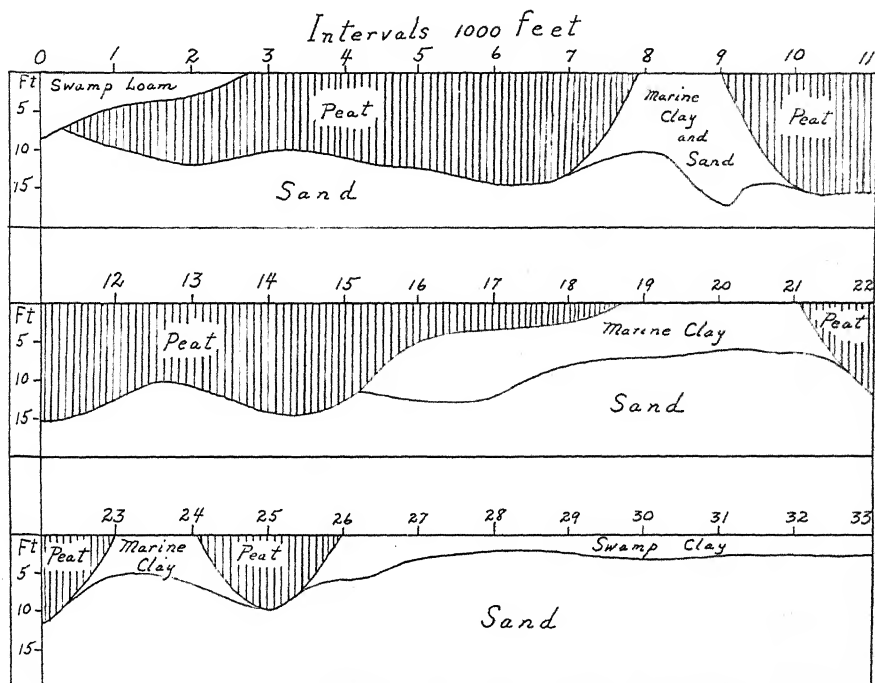
In regard to identification of pollen grains, it is usually easy to place any given grain in the correct family, although some genera not closely related show striking resemblances—*Viola* and *Quercus*, for instance. The identification of genera is more difficult, but frequently possible. In a few cases even the species may be determined with certainty, but in most cases this is impossible.

Certain difficulties in identification present themselves to the student of fossil pollen. It is not easy to free all the grains from the organic material which adheres to them, and frequently minute structures, important in differentiation, are obscured by detritus. Distortion due to folding and collapsing, as well as the different views from which pollen is seen on mounted slides, adds to the difficulty of separating the grains. So far there is no comprehensive manual for identification. The work of Meinke (1927) is probably the best, but it describes only European species, and consequently its usefulness to the American student is limited. Dokturowsky and Kudryachov (1923) describe and illustrate the pollen of the principal European forest trees. Sears (1930) has a key, description, and plates of many important American types. Wodehouse is doing important work on the morphology of pollen grains. His descriptions and plates are very useful for some species. His book which is now in press should help solve many problems of identification of pollen. Lewis and Cocke (1929) illustrated the principal types found in the Dismal Swamp.

Coming to the last aspect, that of preservation of pollen in peat, it can be said that most species are well preserved. A few genera such as *Juniperus*, *Chamaecyparis*, and *Taxodium* are less resistant and often collapse or break to pieces, making differentiation impossible.

In view of the limitations, one might be inclined to question the value of pollen analysis as a method of studying the history of peat bogs. It is probably true, however, "that pollen grains must, on the average, give a truer picture of general vegetation of a neighborhood than the remains of wood and fruits" (Erdtman, 1924, p. 450). Erdtman (1929, p. 112) sums up the values of this method as follows: "The systematic investigation of peat deposits by the modern method of 'pollen analysis' has given us a knowledge

of the immigration, frequency and succession of the trees of European forests during the post-glacial period which would have been thought impossible of attainment a decade ago. Correlated with the study of the first appearance of a given type of tree pollen and its maximum and minimum frequency, has been the study of archaeological remains at the same horizons, and this, together with the evidence of geochronology, has made possible the construction of a fairly trustworthy time scale of the history of our forests. . . ." Sears (1930, p. 96) states, "In spite of these difficulties, the practical results already



Profile of a section of Dismal Swamp, beginning two miles south of Deep Creek and extending 6.2 miles south. Soundings were taken by the Virginia State Highway Commission.

achieved seem to justify the efforts being devoted to pollen statistical analysis."

Even though certain inherent disadvantages are evident, the method of pollen analysis furnishes the most important key to the history of post-glacial plant succession in peat deposits. It gives a fairly accurate picture of the flora existing at the time of deposition of peat at any level.

Topography

The Dismal Swamp district is located on the coast of Virginia and North Carolina, east of the Nansmond Escarpment and extending from the James River to the Albermarle Sound (see map, p. 375).

All of the samples for this study were found to be underlain by an old sea bottom, which is of Pliocene age. The thickness of the peat varied from two to nine feet. The difference in thickness is due to the uneven bottom on which the peat was formed. Table I and figure 1 give a clear picture of the undulating surface on which the peat has been deposited. For a fuller discussion of the topography of this area, see Shaler (1890).

TABLE I. *Table of soundings along State Highway 40 in Norfolk County from two miles south of Deep Creek to Wallaceton. The stations are 1000 feet apart. (Courtesy of State Highway Department.)*

Sta.	Depth of sounding : ft.		Character of soil at different levels				Bottom
245	8	0-6	Swamp loam	6-8	Marine clay		Blue sand
255	10	0-4	"	"	4-10	Peat	Sand
265	12	0-3	"	"	3-12	"	"
275	9	0-9	Peat				"
285	11	0-11	"				"
295	12	0-12	"				"
305	15	0-15	"				"
315	13	0-13	"				"
325	6	0-6	Marine clay and sand				"
335	17	0-17	"	"	"		"
345	14	0-14	Peat				"
355	15	0-15	"				"
365	12	0-12	"				"
375	9	0-9	"				"
385	14	0-14	"				"
395	13	0-13	"				"
405	12	0-4	"	4-12	Marine clay		"
415	12	0-3	"	3-12	"	"	"
425	7	0-2	"	2-7	"	"	"
435	6	0-6	Marine clay				"
445	5	0-5	"				"
455	6	0-6	"				"
465	11	0-11	Peat				"
475	5	0-5	Marine clay				"
485	6	0-6	"				"
495	10	0-10	Peat				"
505	7	0-6	Swamp clay		6-7	Sand	"
515	5	0-3	"	"	3-5	"	"
525	5	0-2	"	"	2-5	"	"
535	5	0-2	"	"	2-5	"	"
545	5	0-3	"	"	3-5	"	"
555	5	0-3	"	"	3-5	"	"
565	5	0-3	"	"	3-5	"	"
575	5	0-3	"	"	3-5	"	"
585	6	0-4	"	"	4-6	"	"
595	5	0-3	"	"	3-5	"	"
605	5	0-3	"	"	3-5	"	"
615	4.5	0-2.5	"	"	2.5-4.5	"	"
625	6	0-4	"	"	4-6	"	"
635	6	0-3	"	"	3-6	"	"
645	5	0-3	"	"	3-5	"	"
655	5	0-2	"	"	3-5	"	"
665	7	0-5	"	"	5-7	"	"
675	8	0-2	"	"	2-8	"	"
685	4	0-4	White clay				"
695	5	0-5	"	"			"
705	5	0-3	Loam		3-5	"	"

Methods of examination

Three sets of samples were taken for this study along the Jericho Ditch at depth intervals of one foot from the surface to the bottom. The depth of the peat at the first boring, two miles from the Norfolk and Western Railroad, Suffolk, Virginia, was two feet; four miles farther it was five feet; and at eight miles from the railroad it was nine feet thick.

Slides for microscopic examination were prepared by the method described previously (Lewis and Cocke, 1929). Diatoms for study were cleaned by boiling in HNO_3 , to which small amounts of potassium chlorate were added.

A total of one thousand pollen grains and spores was counted for each depth. The spores and unknown forms were counted but not included in the percentage tables. The number of each kind of spore and the "unknowns" were recorded as number per thousand. A mechanical stage was used and care taken to avoid repetition in counting. One thousand grains seemed to be quite sufficient to give an accurate picture of the vegetation in existence at the time a layer of peat at any depth was being formed.

There is a difference of opinion among authors as to how many grains it is necessary to count in order to obtain reliable results. Erdtman and other European workers think that a count of from one hundred to two hundred grains is sufficient. Bowman (1931) shows that one hundred grains are too few; he counted from one thousand to eighteen hundred. Sears does not think that estimates made on less than one hundred grains are reliable. Lewis and Cocke (1929) counted eight hundred grains from each depth.

TABLE 2. *Count of each type of pollen by hundreds and five hundreds*

	1	2	3	4	5	6	7	8	9	10	1st 500	2d 500	Total
Nyssa	19	17	14	17	15	15	16	16	17	19	82	83	165
Pinus	15	17	19	17	18	18	12	17	19	13	86	79	165
"Taxodium"	16	19	17	17	13	14	16	17	15	15	82	77	159
Quercus	8	10	10	10	7	7	10	8	14	9	45	48	93
Unknown	7	7	6	5	7	7	5	4	10	6	32	32	64
Bryales	6	5	9	2	3	3	6	7	7	6	25	29	54
Polypodiaceae	6	4	9	8	9	9	11	11	6	5	36	42	78
Gramineae	5	3	3	3	3	3	3	4	2	4	17	16	33
Liquidambar	3	7	0	1	3	3	5	1	1	3	14	13	27
Ilex	4	2	3	2	3	3	4	0	1	3	14	11	25
Fungus	0	2	0	5	0	0	5	2	2	1	7	10	17
Carya	4	3	0	3	1	1	2	1	1	0	11	5	16
Ericaceae	2	1	1	6	2	2	2	0	1	0	12	5	17
Betula	0	2	0	0	3	3	1	1	3	2	5	10	15
Lycopodium	0	0	1	6	1	1	3	0	0	2	8	6	14
Salix	1	0	1	1	2	2	2	2	2	1	5	9	14
Sphagnum	0	0	3	1	1	1	0	4	1	3	5	9	14
Compositae	2	0	1	1	1	1	4	1	1	0	5	7	12
Alnus	1	1	1	0	1	1	1	0	1	1	4	4	8
Herbs	1	0	1	0	1	2	0	0	0	2	3	4	7
Typha	0	0	1	1	0	0	0	0	0	0	2	0	2
Acer	0	0	0	0	0	0	0	1	0	0	0	1	1

Table 2, which gives the count of one depth in hundreds and five hundreds, shows that one hundred grains are too few. In most cases a count of five hundred grains seems to be reliable. However, where the number of species is large, as is the case in the Dismal Swamp, a count of less than one thousand grains is not recommended for accuracy.

Composition of samples

Below twelve feet the substratum is a coarse, compact sand into which the peat borer cannot be forced. From twelve to eight feet inclusive the sand is much finer, with particles averaging about fifty microns in diameter and with an increasing amount of clay. At eleven feet the ash is made up of 88.5 per cent sand and 11.5 per cent clay; at nine feet there is 76.3 per cent sand, 24.7 per cent clay.

From twelve to nine feet inclusive the organic matter is fairly constant, averaging 6.2 per cent. A sharp rise is seen at eight feet, where 22.2 per cent of the material is organic. From seven feet to the surface the organic matter varies from 93.7 per cent (seven feet) to 96.6 per cent (six feet).

Practically no sand is found from seven feet to the surface. The ash in the upper levels is composed of sponge spicules in great abundance, diatom frustules, and unidentifiable flocculent trash.

Diatoms and sponge spicules

A study of the diatoms and sponge spicules in the various levels at Station Three (eight miles from the Norfolk and Western Railroad) has proved most interesting in revealing some of the conditions of the water existing at this place during the history of the swamp.

From the examination it would appear that originally—that is, at the twelve-foot level—the conditions were sandy and salty. This is verified by the many scattered pieces of *Coscinodiscus*, probably *C. obscurus* A. Schmidt, found in the material. Also there were present sponge spicules which closely resemble Bowerbank's descriptions of the skeleton spicules of *Raphyrus griffithsii* Bowerbank and *Hymeniacidon celata* Bowerbank. That during the period, represented by the several inches of material in the sample, fresh water began to appear is verified by the presence of *Cyclotella meneghiana* Grun. and also by fragments of *Pinnularia*.

The samples of material from eleven to seven feet inclusive show that fresh water existed in the swamp during this time. *Pinnularia major* (Kuetz.) W. Smith, *Eunotia lunaris* (Ehr.) Grun., *Eunotia pectinalis* (Kuetz.) Rabenh., *Eunotia major* var. *pulchella* Boyer, *Ephithemia zebra* var. *proboscidea* (Kuetz.) Grun., *Eunotia pectinalis* var. *minor* (Kuetz.) Rabenh., were the main species found in these depths. Many broken pieces of diatoms were found which were too fragmentary for identification, but seem to be mainly of the genera *Eunotia*, *Pinnularia*, and *Stauroneis*. In these depths many amphidiscs typical of the fresh-water sponges were found. From the

variation in the abundance of these forms it would appear that there was comparatively little water at the eleven- and ten-foot levels, but that it gradually increased through the seven-foot level.

At the six-foot level there must have been a great deal of water, at first fresh. Then an invasion of salt water lasted up to and a little above the five-foot level. The diatoms found at this level which indicated salt water were *Coscinodiscus lineatus* Ehr. and several forms of *Schizonema*, among which were forms near *Schizonema corymbosum* Ag. and also *S. parvum* Menegh. There were also present at this level a great number of specimens of *Synedra rumpens* Kuetz. and its varieties. De Toni (1890) states that this species is found only in brackish water. Husted (1909) states that it is found in standing water. Broken pieces of *Coscinodiscus* and *Nitzschia* were found. Also there were present sponge spicules which closely resemble those characteristic of some of the salt-water types.

Just above the five-foot level fresh water returned and remained to the surface. *Eunotia biceps* (Ehr.), *E. lunaris* (Ehr.) Grun., *E. robusta* var. *diadema* (Ehr.) Ralfs, a form close to that of *Navicula fasciata* Lagerst., and also many broken pieces of *Pinnularia* and a few of what may be *Stauroneis* were found. Again the amphidiscs of the fresh-water sponges were present. At the two-foot level two pieces of what seemed to be a *Coscinodiscus* were found. These cannot be explained except that perhaps they were brought in by birds or wind.

From the analysis of this material it appears that water continued to be very abundant from the five- to the four-foot level. There was less water at the four-foot, and still less at the three-foot level. Slightly more water occurred at the two-foot level and decreased thereafter to the surface.

Analysis of pollen

In order to understand the results arrived at in this paper, one must keep in mind the conditions under which the peat was formed. Table 1 and figure 1 give an idea of the conditions of the sea bottom on which the peat was formed. Sometimes narrow hills, as high as fourteen feet, separated depressions which were not more than one thousand feet apart. Thus pollen from trees growing on the higher places might easily be blown to the lower levels.

Evidences which will be presented later seem to indicate that peat was formed in the depressions first and gradually covered the higher places as it continued to be laid down. The picture presented by pollen percentages is not necessarily one of the exact spot where the samples were taken, but represents a composite picture of the general vegetative conditions of that and surrounding areas.

The validity of the conclusions reached by the method of pollen analysis depends on the relation between plant succession and ecological conditions. It is assumed throughout that the importance of hydrophytic types at any

level implies hydrophytic conditions. For example, the occurrence at certain levels of Chytridiaceae cysts in abundance is taken to indicate that there must have been at those levels an abundance of the host plants, which in this case may be various Conjugales. It is concluded from this that at those levels where Chytridiaceae are abundant there must have been an abundance of quiet water suitable to the growth of Conjugales.

Certain pollens and spores offer particular difficulties, and one must always be on guard to catch errors of determination. For instance, *Nyssa* and *Gelsemium* pollen are sufficiently alike to be deceptive. The same is true of *Corylus* and *Betula*. Two mistakes occurring in the paper by Lewis and Cocke (1929) may also be referred to here. First, the pollen described and illustrated as *Castalia* (1929, pl. 5, fig. 26) has been found to be *Nuphar*. Second, the spores illustrated to represent Bryales (1929, pl. 3, fig. 1, 2) proved on more careful study to be a fungus (one of the Ustilagineae) sporulating within the moss capsule. It is this peculiar habitat of *Ustilago* which caused early workers to describe two kinds of spores in *Sphagnum*.

At each level there are a number of grains which cannot be identified. Some are perfect grains of which we have insufficient knowledge. These are believed to represent herbs, since it has been possible for us to become familiar with most of the trees and shrubs of the area. Other apparently perfect grains are so obscured by trash and organic matter that they are unrecognizable. In well-decomposed peat it is sometimes impossible to clear the grains sufficiently for identification.

There are also many disfigured grains which include all types of pollen. Some of these are fragile grains with thin walls which became crushed or deformed under the conditions of peat formation. *Taxodium*, *Chamaecyparis*, and *Juniperus* are good examples of such grains. They are somewhat similar in fresh condition, and their collapse during fossilization often makes it impossible to distinguish them. This group can be distinguished from other genera, however. In this paper the term "*Taxodium*" is used to refer to grains identified as belonging to this group. This does not indicate that all grains so labeled belong to the genus *Taxodium*. Some may represent *Chamaecyparis* or even *Juniperus*; but since *Juniperus* is not found today in the area studied and *Chamaecyparis* is unimportant, while *Taxodium* occurs abundantly, the term "*Taxodium*" has been selected to include all these grains.

Unidentifiable fragments of grains include a variety of genera. They are usually numerous wherever sand is present mixed with the peat.

Meinke (1927, p. 399) points out that "scientific exactness demands that all the pollen-forms if possible should be considered." While this is undoubtedly true, the grains which can be definitely identified give a fairly accurate picture of the type of vegetation existing in the bog at any level.

RESULTS AND CONCLUSIONS

Borings were studied from three stations along the Jericho Ditch between the Norfolk and Western Railroad tracks near Suffolk and Lake Drummond. Station 1 is two miles, station 2 six miles, and station 3 eight miles south of the railroad.

TABLE 3. *Station 1. Percentage of fossil pollen at one-foot intervals*

Trees and shrubs	Depth in feet		
	2	1	Surface
"Taxodium"	21.4	14.7	9.5
Nyssa	18.8	20.2	18.3
Quercus	4.6	8.3	9.7
Accr	4.9	7.1	9.6
Liquidambar	3.0	8.0	12.8
Salix	0.8	1.8	0.4
Pinus	13.8	15.6	14.0
Alnus	1.6	0.7	0.2
Betula	2.6	0.4	0.2
Carya	0.9	1.2	1.5
Ilex	2.0	2.4	0.2
Ericaceae	1.6	2.3	0.5
Fagus		2.6	3.1
Platanus		1.2	1.9
Myrica		1.2	1.3
<i>Total of trees and shrubs</i>	76.0	87.7	83.2
Gramineae	16.1	8.1	9.8
Cyperaceae	4.5	2.2	0.4
<i>Total of grasses and sedges</i>	20.6	10.3	10.2
Herbs	3.4	2.0	6.6
<i>Number of spores and unknown grains per thousand pollen grains</i>			
Polypodiaceae	40	85	41
Bryales	25	23	40
Fungi	9	11	3
Lycopods		1	6
Sphagnum			2
Unknown	56	40	54

Station 1

The shallowness of the peat (only two feet) indicates that it had been formed on one of the elevations of the old sea bottom. The pollen count at the lowest level, two feet from the surface, given in table 3, shows that at the time of the deposition of the peat at that level a well-established forest with a considerable amount of open space was the type of vegetation. The pollen of woody plants makes up 76 per cent of the total number of grains at this level. Grasses and sedges occur in appreciable amounts in the open spaces. Herbs are also well represented at this level.

"*Taxodium*," which is the dominant forest type, makes up 21.4 per cent of the total pollen and 28 per cent of the tree pollen at this depth. *Nyssa* and

Pinus are also important constituents of the forest. The high percentages of *Quercus*, 4.6, and especially of *Acer*, 4.9, are unexpected. The absence of *Sphagnum* and the low percentage of *Salix* and *Alnus*, along with the relatively high percentage of other forest types, indicate a dry substratum.

The maximum arboreal vegetation for this area is reached at the one-foot level. The percentage of tree and shrub pollen has increased from 76.0 to 87.7, with a corresponding decrease in the grass-sedge type of vegetation from 20.6 to 10.3. *Nyssa* has replaced "*Taxodium*" as the dominant forest type. *Quercus*, *Ilex*, *Liquidambar*, *Acer*, and *Carya* show substantial increases, indicating a richer type of vegetation of the gum-maple-oak type found there today. *Pinus* shows a small increase. *Myrica*, *Platanus*, and *Fagus* come in at this level.

Grasses, sedges, and herbs are reduced to the normal incidence of these types.

The polypods show a marked increase; the forest type of ferns, represented by *Asplenium* and *Aspidium*, is the most abundant type encountered. Lycopods occur for the first time at this level.

At the surface influences of clearing are noticed, although they are less evident than at some other places in the Swamp. The percentage of tree pollen decreases from 87.7 to 83.2, with a slight increase in grasses and sedges and herbs. The influence of cutting is not shown so clearly by the small decrease in trees and shrubs as by the kind of trees which show decreases and increases. "*Taxodium*" shows the greatest loss, while *Nyssa* also shows a small loss. These two are probably used more as lumber than any other trees of this area. On the other hand, *Quercus*, *Liquidambar*, *Acer*, *Carya*, *Platanus*, *Myrica*, and *Fagus*, which are common second-growth trees of this vicinity now, all show noticeable gains. The slight decline of *Ilex*, *Alnus*, *Betula*, *Salix*, and the heaths and the dominance of *Nyssa* are in keeping with the conditions of the present-day flora of this area.

The polypods show a decided drop, while the number of lycopod spores found is slightly higher than at the one-foot level.

The occurrence for the first time of *Sphagnum* is noteworthy, and supports the conclusions of Lewis and Cocke (1929, p. 49) that it "cannot be responsible for the upbuilding of the peat level."

Station 2

The samples for this particular locality were taken six miles from Suffolk, on the Jericho Ditch (see map, p. 375). The borings were made in a closed but not dense forest, composed principally of second-growth trees, such as *Nyssa*, *Ilex*, *Quercus*, *Acer*, *Liquidambar*, and *Platanus*. *Pinus* and "*Taxodium*" were also present, but these types were represented by the older trees rather than new growth. Grasses, sedges, herbs, mosses, ferns, and some *Sphagnum* were present in noticeable quantities.

The presence of woody plants in relatively high percentage, 55.7 at the bottom of the peat, indicates an intermediate stage of the forest at this level. On the other hand, grasses and sedges make up 40.1 per cent of the total pollen. This indicates, of course, much open land covered by the grass-sedge type of vegetation.

TABLE 4. Station 2. Percentage of fossil pollen at one-foot intervals

Trees and shrubs	Depth in feet					
	5	4	3	2	1	Surface
"Taxodium"	14.8	20.8	42.2	32.3	25.8	7.0
Nyssa	3.4	9.2	9.5	16.7	23.6	18.2
Quercus	4.9	6.1	8.6	7.4	5.1	7.9
Acer	1.4	5.1	2.8	3.6	6.7	5.4
Liquidambar	3.6	3.5	2.2	2.6	1.2	3.2
Salix	6.8	4.1	1.4	0.7	0.4	1.9
Pinus	11.9	15.7	9.7	13.1	15.6	17.0
Alnus	4.7	1.3	0.3	0.9	0.7	0.7
Betula	2.1	1.2	0.6	2.1	1.2	0.7
Carya	0.6	2.5	2.3	1.3		0.9
Ilex	1.5	1.5	6.0	7.2	6.3	8.5
Ericaceae		1.2	2.3	5.0	5.7	4.2
Fagus				0.6		
Platanus			0.7	0.3	1.3	2.7
Myrica					0.5	0.7
Total of trees and shrubs	55.7	72.2	88.6	93.8	94.1	79.0
Gramineae	27.4	8.6	3.5	2.5	1.7	7.4
Cyperaceae	12.7	8.2	0.7		1.3	3.0
Total of grasses and sedges	40.1	16.8	4.2	2.5	3.0	10.4
Herbs	4.2	11.0	7.2	3.7	2.9	10.6
Number of spores and unknown grains per thousand pollen grains						
Polypodiaceae	3	8	11	10	17	14
Bryales	14	30	51	72	80	131
Fungi	84	46	31	21	27	60
Lycopodium			3	2	6	6
Sphagnum	21	23	41	27	15	7
Unknown	154	75	29	30	43	89
Chytridiaceae	90	116				

The occurrence of *Sphagnum* and the fact that *Salix* and *Alnus* reach their maximum at this level point to wet conditions. Diatoms of the fresh-water species and Chytridiaceae cysts are also present.

"*Taxodium*" is the dominant forest type at this level. The other forest constituents, *Pinus*, *Nyssa*, *Quercus*, *Ilex*, *Liquidambar*, *Acer*, *Carya*, *Alnus*, *Betula*, and *Salix*, are present in sufficient abundance to indicate that conditions were favorable for their growth.

A study of table 4 shows that rapid progress is being made toward a closed forest at the four-foot level. The percentage of trees and shrubs increases from 55.7 to 72.2, while the grasses and sedges have been reduced by more than half of their former percentages, from 40.1 to 16.8. Practically all types

of forest trees show substantial gains with the exception of *Alnus* and *Salix*, which are on the decline. This points to a general drying out and more favorable growing conditions.

"*Taxodium*" continues as the most important tree. It also shows the greatest gain, 6.0 points, indicating that it was better adapted to the conditions existing at that time than the other members of the forest population. The gain of 5.8 points by *Nyssa* shows that it also is developing rapidly.

Bryales, polypods, and *Sphagnum* each show some increase at this depth. Fungi drop considerably.

Chytridiaceae and diatoms remain in the picture, indicating the presence of pools of fresh water.

One new family, Ericaceae, occurs at this level.

The three-foot level is particularly interesting. The total arboreal vegetation increases 16.4 points over the depth below. The increase in "*Taxodium*" alone, 21.4 points, exceeds this gain. The edaphic conditions suitable for the development of "*Taxodium*," which reaches its maximum at this time, were optimum at this level. In spite of the remarkable increase of "*Taxodium*," the genera *Nyssa*, *Quercus*, *Ilex*, and the Ericaceae were able to gain some ground. All other forest types show slight decreases. It is interesting to note that *Sphagnum* reaches its maximum along with "*Taxodium*."

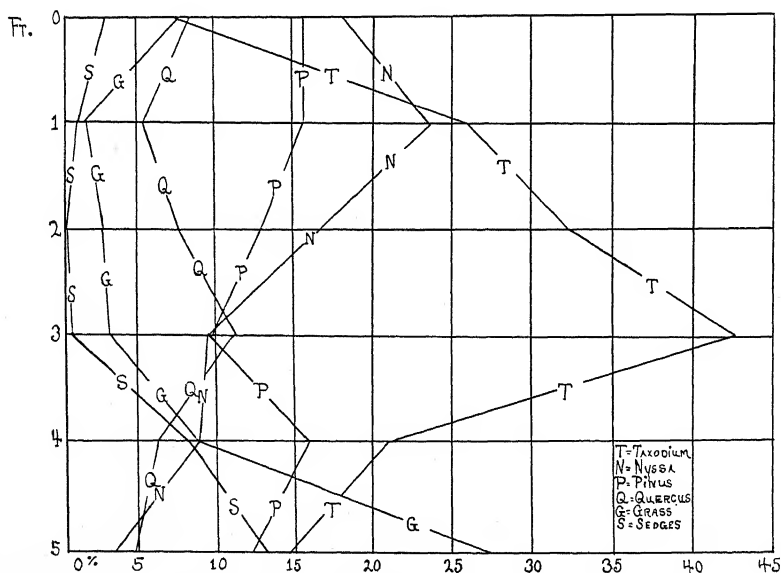


Chart 1. The percentage of certain important genera at station 2.

At the time of the deposition of peat at this level, a practically closed forest, dominated by "*Taxodium*," was the type of vegetation. Grasses and sedges are unimportant at this level, the total percentage of the two being

only 4.2. Bryales show an increase, while the polypods decrease. *Lycopodium* and *Platanus* are new at this depth. The changes in vegetation, accompanied by a great decrease of Chytridiaceae, indicate an increasingly dry substratum.

The trees reach their maximum state of development at the two-foot level. At this depth they constitute 93.8 per cent of the total vegetation. The absence of sedges and the low percentage of grasses, 2.5, indicate that there are practically no open spaces. "*Taxodium*" is still the dominant forest tree, although the conditions so favorable for its growth at the three-foot level are now shifting in favor of other forest forms. This is shown by the sharp decline in "*Taxodium*" from 42.2 per cent to 32.3 per cent and the gains noticed especially in *Nyssa*, with smaller gains in *Pinus*, *Ilex*, *Liquidambar*, *Acer*, Ericaceae, *Betula*, and *Alnus*. *Quercus*, *Salix*, *Carya*, and *Platanus* show slight decreases.

The mosses are more abundant than at any previous level. *Sphagnum*, polypods, and lycopods show a small loss.

One new genus, *Fagus*, occurs at this level.

The changes which began between the two- and three-foot level continue to express themselves at the one-foot level. "*Taxodium*" continues to decrease, while *Nyssa*, *Acer*, Ericaceae, *Platanus*, and *Myrica* are becoming more plentiful, pointing toward the gum-maple forest type existing there at present. Since *Ilex* is insect-pollinated, its decline is unimportant. The decrease noticed in *Quercus* and *Liquidambar* is unexpected, especially the latter, since it is an important constituent of the present flora of this region.

The effect of clearing on the vegetation of this part of the Swamp is shown at the surface. The percentage of trees and shrubs drops from 94.1 per cent to 79.0 per cent. The increase in total percentage of grasses and sedges is not so great as would be expected, although it does indicate the presence of more open spaces. "*Taxodium*" and *Nyssa* suffer the greatest loss at this level, as is to be expected in view of their value as lumber trees and the extent to which they have been logged. The great decrease in "*Taxodium*" is probably due to changing edaphic conditions which have been mentioned as well as to cutting.

The young growth of *Quercus*, *Ilex*, *Liquidambar*, *Carya*, *Platanus*, and *Myrica* existing in this area today accounts for their increase. *Pinus* and *Acer* show minor decreases, as do the Ericaceae. *Betula* and *Alnus* are rare in this vicinity at the present time, so that their decline is expected.

The spores of Bryales are considerably more numerous, merely indicative of the luxuriant growth of moss there now. Both polypods and lycopods decrease, which is also in keeping with present floral conditions.

Summary of results of the study of peat samples taken at station 2. The peat at this location was five feet thick, underlain by marine sand and clay. At the beginning of deposition of peat in this area, a fairly well-established forest, dominated by "*Taxodium*," with much open land was the type of

vegetation. The development was rapid, becoming a closed forest type at the three-foot level and remaining so through the one-foot level. At the surface evidences of clearing are seen.

Chart 1 shows that "*Taxodium*," which was the dominant forest type at the bottom, developed rapidly to the three-foot level and then showed a steady decline. The *Nyssa* curve shows a steady development until it is checked by clearing at the surface.

TABLE 5. Station 3. Percentage of fossil pollen at one-foot intervals

Trees and shrubs	Depth in feet									Surface
	9	8	7	6	5	4	3	2	1	
" <i>Taxodium</i> "		6.0	11.0	4.9	8.4	20.1	31.5	34.4	16.0	11.9
<i>Nyssa</i>	4.3	2.6	6.0	3.8	10.2	22.2	26.0	23.2	34.3	15.5
<i>Quercus</i>	4.9	8.6	22.6	7.2	7.8	10.2	4.4	8.8	4.8	5.3
<i>Acer</i>	6.1	7.0	15.8	3.6	2.0	2.8	2.2	4.1	2.6	5.6
<i>Liquidambar</i>			1.3	7.4	4.3	3.4	3.1	1.0	1.9	3.6
<i>Salix</i>	5.9	1.9	4.2	12.5	19.5	1.8	0.8	1.8	1.5	
<i>Pinus</i>	4.0	7.2	6.4	19.2	23.6	21.5	16.6	11.4	21.4	14.3
<i>Alnus</i>	2.4	4.3	0.2	0.3	0.3	1.0	0.1	0.8		1.0
<i>Betula</i>	2.4	0.7	3.5	1.6	0.9	1.7	1.0	0.5	0.6	0.8
<i>Carya</i>			2.0	0.8	2.5	2.3	1.3	0.7	1.8	3.0
<i>Ilex</i>		0.7	1.3		2.8	3.3	9.0	9.2	5.3	4.1
<i>Ericaceae</i>			0.5	0.5	1.5	2.2	2.0	1.1	4.2	2.2
<i>Fagus</i>				0.9	0.1					
<i>Platanus</i>					0.3			0.1		
<i>Myrica</i>		0.4			0.8		0.3	0.1		
<i>Benzoin</i>		0.3	0.1							
<i>Carpinus</i>								0.1		
<i>Juglans</i>					0.2					
<i>Total of trees and shrubs</i>	30.0	39.7	74.9	62.7	85.2	92.5	98.3	97.3	94.4	67.5
<i>Gramineae</i>	45.0	36.2	14.7	19.8	7.4	4.5	0.8	1.0	1.7	27.3
<i>Cyperaceae</i>	11.9	12.7	0.8	2.7	1.3			0.2	0.7	
<i>Total of grasses and sedges</i>	56.9	48.9	15.5	22.5	8.7	4.5	0.8	1.2	2.4	27.3
<i>Herbs</i>										
<i>Nuphar</i>	5.0	4.5	2.5	7.1	0.8					
<i>Typha</i>	3.1				0.1	0.5	0.2			0.3
<i>Compositae</i>	2.5	2.5	2.2	1.0	1.5	1.6	0.6	0.6	0.6	3.7
<i>Umbelliferae</i>	1.0	2.2	1.3	3.1	0.8			0.2	1.4	0.4
<i>Other herbs</i>	1.5	2.2	3.6	3.6	2.9	0.9	0.1	0.7	1.2	0.8
<i>Number of spores and unknown grains per thousand pollen grains</i>										
<i>Polypodiaceae</i>	39	6	14	2	38	79	18	15	74	2
<i>Bryales</i>		11	60	62	40	57	78	47	32	57
<i>Fungi</i>	86	60	68	54	13	21	21	12	16	31
<i>Lycopodium</i>				2	18	15	3	3	13	8
<i>Sphagnum</i>			3	2	5	14	34	29	10	
<i>Osmunda</i>				2	3					
<i>Unknown</i>	205	99	51	74	50	67	40	17	25	62
<i>Chytridiaceae</i>	81	98	204	73						

Station 3

The general picture of vegetative conditions at the nine-foot level when peat began to form at this location is similar to that found by Lewis and Cocke

(1929) in another area of the Swamp where the peat was approximately of the same thickness. The high percentage of grasses and sedges, 56.9, with a low percentage of woody plants, 30.0, proves the existence of an open marsh type of vegetation. Even though *Nuphar* is insect-pollinated and the percentage of pollen is not reliable as an indication of its abundance at any given level, a percentage of 5.0 indicates that it must have been present in appreciable amounts. Its presence, along with that of diatoms and Chytridiaceae, and the relatively high percentage of *Typha*, 3.1, leads to the conclusion that there was considerable water present. That the water was fresh is shown by the presence of *Typha latifolia* and the occurrence of *Nuphar*. A study of the diatoms supports this conclusion.

Only seven types of trees were present at this level, and these in percentages sufficiently low to be accounted for by blowing in from the higher places. This is probably what happened in the case of *Acer*, *Quercus*, *Nyssa*, and *Pinus*. The other types, *Alnus*, *Betula*, and *Salix*, could have been growing there under the existing conditions.

At the next level progressive changes in the development of vegetation are evident. The total percentage of tree and shrub pollen increases from 30.0 to 39.7. There is a general increase in all forest types except *Nyssa*, *Salix*, and *Betula*. The number of genera also increases. "*Taxodium*," *Ilex*, *Myrica*, and *Benzoin* are new forest types at this depth. Bryales and herbs also occur for the first time at this level. The occurrence of *Ilex*, an insect-pollinated species, shows that it was actually growing in this area at the time the peat was being formed. Its presence is proof that conditions were favorable for the growth of the other genera listed in table 5.

The fact that the percentage of grasses and sedges remains high is an indication that there is still a considerable amount of open land covered by the grass-sedge type of vegetation.

Water is still abundant at this level, as is shown by the continued presence of *Nuphar*, diatoms, and Chytridiaceae cysts. *Typha* has disappeared, however.

The percentage and types of pollen found at the seven-foot level indicate that a beginning of a permanent forest is well under way. The percentage of woody plants increases enormously, from 39.7 to 74.9, with every important genus showing substantial gains, except *Pinus* and *Alnus*, which are evidently being choked out. *Quercus* and *Acer* show the greatest gains, the former with 14.0 points increase and the latter 8.8 points. "*Taxodium*" and *Nyssa* also exhibit marked advances over the depth below.

The occurrence of three new forest types, *Carya*, *Liquidambar*, and *Ericaceae*, is important. *Sphagnum* also comes in at this level.

The grasses and sedges show a drop in keeping with the tremendous increase of trees. They diminish from 48.9 per cent to 15.5 per cent. *Nuphar* is also a loser at this depth. Diatoms are still present and Chytridiaceae show a slight increase.

The general conditions of the Swamp as presented by the pollen remains of the flora of this level show the formation of a well-established forest populated by thirteen genera of forest trees. Much of the open land formerly covered with grasses and sedges has been encroached on by the forest trees.

At the six-foot level the progressive development of the forest seen at the seven-foot level is upset. The arboreal vegetation which made up 74.9 per cent of the total vegetation is reduced to 62.7 per cent. Grasses and sedges increase from 15.5 per cent to 22.5 per cent. Whereas, at the previous depth most of the trees showed marked gains, the reverse is true at the six-foot level, *Pinus*, *Liquidambar*, *Salix*, and *Alnus* being the only trees to show any gain. *Ilex* disappears entirely at this level.

The relatively high percentage of *Nuphar* and *Salix* indicates wet conditions in the Swamp at this time.

This period of depression in the normal development is particularly interesting, since it corresponds to a similar condition found by Lewis and Cocke (1929) in their study of peat from near Wallaceton. The unfavorable period described by them occurred at the seven-foot level, while the present one is evident at six feet. However, the thickness of the peat in the former case was ten feet, while in the latter it is only nine feet, so that in both cases the check came when three feet of peat had accumulated.

The causes of the retrogression noted at these two localities of the Swamp are evidently the same. Lewis and Cocke (1929, p. 46-47) suggest as a possible cause a sinking of the land level accompanied by an increase in standing water. The drop in the total percentage of trees and shrubs, accompanied by the increase in grasses and sedges, is significant. Among the trees notable gains are shown by *Pinus* and *Salix* (about 200 per cent), while the general loss in other trees is shown most strikingly by *Quercus* (about 67 per cent) and *Acer* (about 75 per cent). This great increase in hydrophytic trees, with corresponding decreases in the more mesophytic genera, points to the correctness of the conclusion stated above, which is further supported by the increase in *Nuphar* (about 195 per cent). The decrease in Chytridiaceae is progressive from the seven-foot level, and hints at the possibility that brackish conditions may have prevailed. The occurrence of *Nuphar* and the fact that Chytridiaceae are still common, even though less abundant, show that the surface water could not have been more than brackish.

The study of the diatoms discussed above supports the conclusion that an increase of water in the Swamp at this time was the cause of this retrogression. This study indicated that the amount of water gradually decreased from the ten-foot level to the seven-foot level. At the six-foot level there was much water present, which was at first fresh, then salty. It might be noted that the same conclusions were arrived at from the study of the diatoms and the pollen remains by separate authors working independently.

The greater severity of the check noted at Wallaceton than that shown by the Jericho Ditch material is accounted for by the fact that Wallaceton is

lower by at least 7 feet than the area north of Lake Drummond, so that the effect of any subsidence would be more severely felt at Wallaceton.

The unfavorable conditions prevailing at the six-foot level seem to have disappeared at the next depth. The total of forest types shows a marked jump from 62.7 per cent to 85.2 per cent. At the same time, the percentage of grasses and sedges has been reduced considerably, from 22.5 to 8.7. The decrease in *Nuphar* from 7.1 per cent to 0.8 per cent accompanied by decrease in diatoms, together with the appearance of *Juglans* and *Platanus* and the reappearance of *Myrica*, indicates physiologically dryer and more favorable growing conditions for the forest trees. The increase in *Salix* does not affect this conclusion, since it had become well established at the six-foot level, and the other constituents of the forest had not become sufficiently well established to choke it out as they later do.

The percentage of trees and shrubs continues to increase at the four-foot level. The woody plants now make up 92.5 per cent of the total flora. A decided progression toward a climax forest is shown at this level. The loss in *Salix* from 19.5 per cent to 1.8 per cent and the substantial gains noted in *Nyssa*, *Pinus*, and "*Taxodium*" indicate that *Salix* is being crowded out by these more robust members of the forest population. *Platanus*, *Myrica*, *Fagus*, *Benzoin*, and *Juglans* are absent from this depth, but none of them has played an important part in the history of the vegetation at any previous level.

The absence of sedges and *Nuphar* and the decrease in grasses and *Salix* indicate progressive tendencies in the type of vegetation. Mosses and ferns, both common forest types, show gains at this level. The large increase in "*Taxodium*" and *Nyssa* is indicative of conditions favorable for the development of the cypress-gum type of forest.

Development toward a climax forest type of vegetation is evident at the three-foot level. The arboreal vegetation constitutes 98.3 per cent of the total vegetation at this level. "*Taxodium*," *Nyssa*, and *Ilex* each show important gains. All other forest types show losses. This is an expression of the tendency noted at the four-foot level, pointing toward a cypress-gum type of forest. Grass is reduced to a very low percentage, 0.8, and sedges are absent, which shows that there are practically no open spaces in the area at this time.

It is interesting to note that *Sphagnum* and the Bryales reach their maximum at this level.

Another change in growing conditions is foreshadowed at the two-foot depth. Although "*Taxodium*" is the dominant forest type, with 34.4 per cent of the total pollen, nevertheless the high percentage of *Nyssa*, 23.2, and the increase in *Quercus*, *Ilex*, and *Platanus* indicate increasingly mesophytic conditions, favoring the gum-oak-maple type of forest. The herbaceous constituents of the vegetation continue unimportant at this level.

The conditions foreshadowed at the previous level are evident at the one-

foot depth. "*Taxodium*" has been reduced from 34.4 per cent to 16.0 per cent, while *Nyssa* has increased from 23.2 per cent to 34.3 per cent. *Liquidambar*, *Carya*, *Betula*, and *Pinus* are on the increase. Even though *Quercus*, *Acer*, and *Ilex* show losses, they are still present in quantities large enough to be in keeping with the general picture presented. The great decrease in *Sphagnum* also points to more mesophytic conditions.

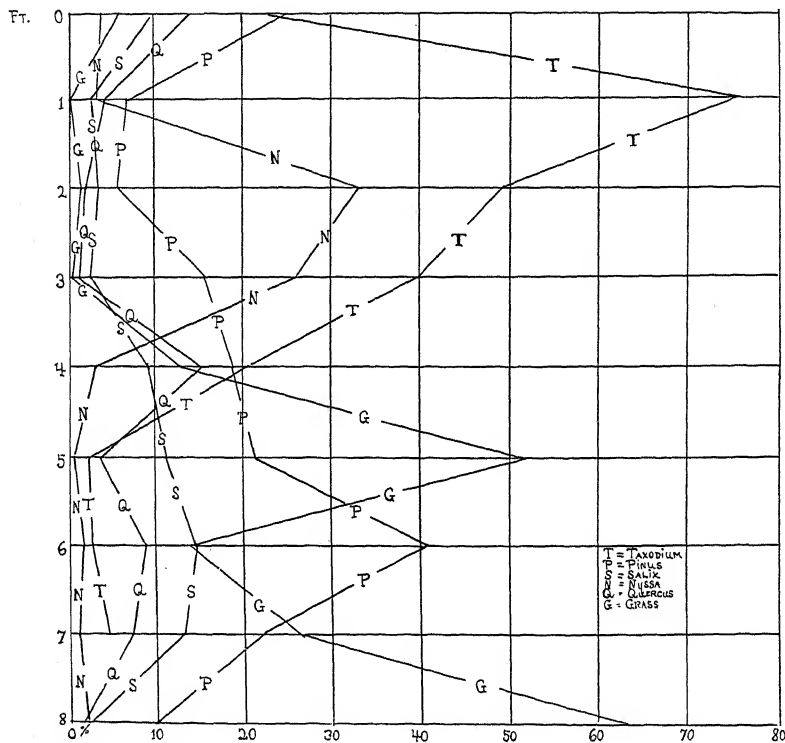


Chart 2. The percentage of certain important genera at station 3.

The picture exhibited at the surface is essentially the same as that found elsewhere in the Swamp, where the influence of cutting is always evident. There is a sharp decline in total percentage of trees from 94.4 to 67.5, with a correspondingly high increase in the percentage of grasses from 2.4 to 23.7. "*Taxodium*," *Nyssa*, and *Pinus* show the greatest decrease. *Quercus*, *Liquidambar*, and *Acer*, which are important second-growth trees there now, show increases.

Summary of the study of peat samples from station 3. The general picture of the history of the vegetation is essentially the same as that described by Lewis and Cocke (1929) in their study of another area of the Swamp. It shows a steady development from a grass-sedge meadow to a climax forest. At the six-foot level the development was checked, probably by subsidence

of land accompanied by an invasion of salt water, killing out much of the arboreal vegetation. The development of "*Taxodium*" parallels that of *Nyssa* very closely from the bottom to the three-foot level. "*Taxodium*" is dominant at this and reaches its maximum at the next level. *Nyssa* is the most important type at the one-foot level and on the surface.

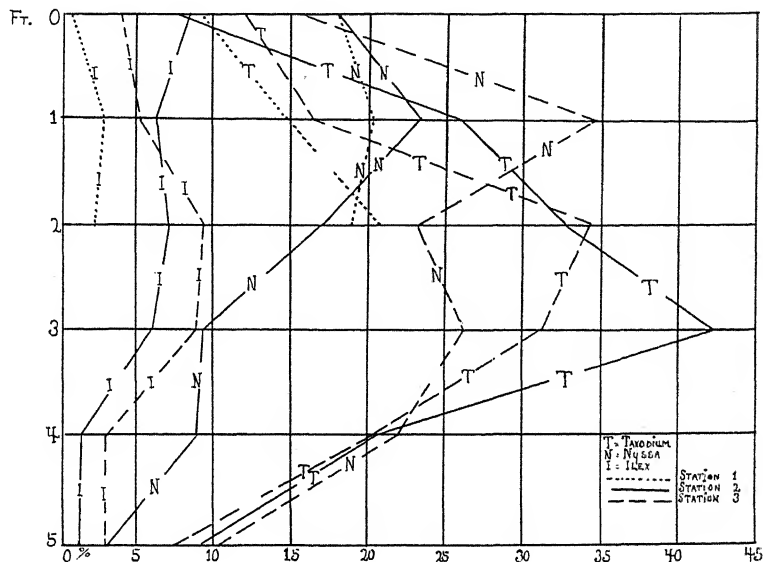


Chart 3. The percentage of "*Taxodium*," *Nyssa*, and *Ilex* found in the upper five feet of the three sets of samples.

SUMMARY

On Jericho Ditch six miles from Suffolk the peat was five feet thick. While the vegetational picture at the bottom (five feet) shows some similarity to the bottom depth of the nine-foot boring, the differences are more striking. Much less open space and more healthy growing conditions for forest types are indicated. In fact, the conditions are more like the five-foot level of the nine-foot boring. Chart 3 shows how closely the upper five feet of the two borings resemble each other.

The peat was only two feet thick on the Jericho Ditch two miles from Suffolk. The two-foot level showed a much richer vegetation and less open land than have been found at the bottom of the peat anywhere else in the Swamp. The picture presented by these two feet is similar to the upper two feet of the other borings.

The striking similarity shown by corresponding levels at different places in the Swamp supports the conclusion that the peat was formed in the lower places first and gradually covered the elevated areas as it continued to be formed. The minor differences can easily be accounted for by different edaphic conditions existing in the various localities.

The history of the vegetation on the Jericho Ditch eight miles from Suffolk, where the peat was nine feet thick, coincides in a general way with the history of the area around Wallaceton. Both developed from an open marsh covered with grasses and sedges to a closed forest type. In each case the

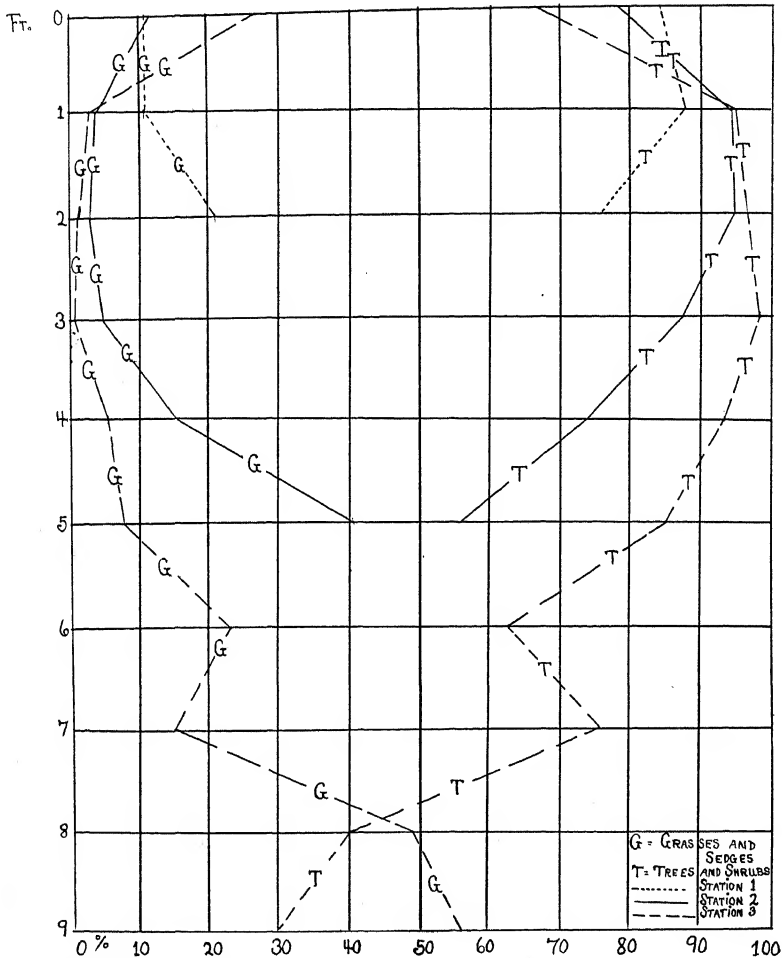


Chart 4. The percentage of trees and shrubs, and grasses and sedges at the various levels of the three stations.

development was arrested at a low level—around Wallaceton at the seven-foot level and on the Jericho Ditch at the six-foot level. Land subsidence, accompanied by a rise of water in the Swamp, is considered to be the cause of the arrested development. Present evidence indicates that this water, at first fresh, became salt and then again fresh.

From the level of this depression to the surface the development of these two areas parallels each other very closely, with only expected minor differ-

ences, among which should be noted the presence of *Carya* north of Lake Drummond. This genus was not noted in the earlier studies. *Quercus* plays a much more important part along the Jericho Ditch than it does in the Wallaceon area.

The writers wish to acknowledge gratefully the valuable assistance rendered in the preparation of this paper by Dr. Paul W. Bowman, of George Washington University, Dr. P. B. Sears, of the University of Oklahoma, Dr. B. D. Reynolds, of the University of Virginia, and the Virginia Forestry and Virginia State Highway Departments.

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CORDAITEAN WOOD FROM THE PENNSYLVANIAN OF KANSAS¹

WALDO EDWARD STEIDTMANN

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Recently two samples of Pennsylvanian, silicified wood, from Kansas, were placed at the disposal of the writer.² One of the samples is accompanied by full collection data and was found in Anderson County, Kansas, by M. K. Elias of the Kansas State Geological Survey. The other sample had a label reading "Silicified wood, Coffeyville, Potato Creek, Montgomery County, Kansas." According to the Bulletin of the Geological Survey of Kansas (Moore and Haynes, 1917), Coffeyville lies in the area where there are outcrops of the Marmaton group of the lower Pennsylvanian. Both specimens were in a collection of unstudied material in the Museum of Paleontology of the University of Michigan.

Similar material was described by Penhallow (1900) when he undertook the revision of Dawson's collection of petrified woods. In that collection there were two specimens collected by Professor Prosser in Chase County, Kansas, from the base of the Permian. One of the specimens Penhallow assigned provisionally to *Dadoxylon* because of its poor preservation and because it showed no definite relationship to any other genus. The other he placed in *Cordaites*. In both instances the description is very meager and without illustrations, so that in these respects the original publication is of little value. Through the courtesy of Dr. T. H. Clark of the Peter Redpath Museum, McGill University, the type material was loaned for comparison. The slides labeled *Dadoxylon prosseri*, to which the Anderson County material seems most nearly related, are useless for accurate comparison. The slides labeled *Cordaites illinoiseuse*, representing sections from three specimens, one of them originally labeled by Dawson (1863) as *Dadoxylon missouriense*, differ somewhat from one another, and materially from the specimens here to be described, and will consequently be disregarded in the present paper. The Coffeyville specimen bears some resemblance to and is apparently quite closely related to *Cordaites materiaram* as described by Dawson (1863) and Penhallow (1900).

A number of specimens of Cordaitean wood have been described from

¹ Papers from the Department of Botany and Herbarium of the University of Michigan, No. 415.

² The writer obtained access to the material through the courtesy of Dr. C. A. Arnold of the Department of Botany and the Museum of Paleontology of the University of Michigan.

various parts of the world. In many of these the primary structure has been absent, which has prohibited the establishment of their exact generic affinity. Because of the absence of primary structures, various criteria of secondary wood have been used in an attempt to arrive at specific determinations. The presence and absence of growth rings were used by Grand'Eury (1877) and Kraus (1864), while Dawson (1863) even founded a species on their presence. Obviously there are objections to this because of the difference of opinion as to what constitutes a growth ring and how well-defined this ring must be before it may be recognized as such. Goldring (1921) has even placed an Oklahoma specimen, with growth rings, in the same genus and species with one of Penhallow's from Prince Edward Island, without growth rings. At any rate, there is no reference made to their presence in his description.

Attempts have been made to utilize cell measurements for identification purposes. Such measurements are used to some extent in the recognition of commercial species of modern woods, but are considered of dubious or only of regional value unless, as Desch (1932) indicates, they are taken from a large number of specimens.

The seriation and other characteristics manifested by the pits in the tracheids are used extensively as a basis for classification of secondary wood. Von Mohl (1861-1862) and Schacht (1861-1862) have found that there may be considerable variation between the wood of a root and a stem of the same plant. Holden (1917) has pointed out a marked difference between the pitting of an Indian *Pinus* and the same genus from the West. Apparently, then, pitting, as a generic characteristic, must be used with some caution.

This leaves little more than the seriation and the height of the rays, the length of the ray cells, and their lateral pitting to be considered. Since the material here to be described is comparatively well preserved but consists of secondary structure only, all the above criteria for identification will be used and emphasized according to their relative value.

Carboniferous wood of the *Dadoxylon* type is relatively simple in structure when compared with modern gymnosperms. There may be growth rings present but they are never of the kind which exhibit broad, unmistakable summer wood. Resin ducts or resin canal systems are absent. The wood rays are usually narrow and variable in height and never fusiform. The ray cells are all of one kind and in general manifest the same type of lateral pitting. "Bars of Sanio" are generally not found separating the somewhat complex araucarian arrangement of pits. The wood may be characterized as simple and compact in nature.

Dadoxylon douglasense, sp. nov. Tracheids about 58μ in radial diameter, often much less in lateral diameter; somewhat rounded, walls less than 12μ thick. Pits in 1-2 rows, alternating when in 2 rows, rarely appearing hexagonal when crowded, 12.5μ in diameter, orifice lenticular, $\frac{1}{3}$ the diameter of the pit. Rays numerous, 1-40 cells high, uniseriate, or biseriate in part;

in radial view cells extending over $1\frac{1}{2}$ –7 tracheids; the lateral walls of the ray cells and adjoining tracheids displaying 1–2 pits. Marginal ray cells often higher than ray cells and often with 3–4 pits.

Horizon, base of Douglas group, upper Pennsylvanian. Locality, near Garnett, Anderson County, Kansas. Type no. 15112, Museum of Paleontology, University of Michigan.

The specimen is a fragment of what was evidently a large trunk. It measures 20 cm. in length and 10 cm. in diameter and represents only a part of the radius of the stem.

A transverse thin section, held to the light, indicates a gymnospermous wood with indefinite zones which might be interpreted as growth rings. Under microscopical examination these zones are found to be mostly the result of disintegration followed by partial crushing. Nevertheless, there are zones of large cells which alternate with narrow zones of small cells. These regions are discontinuous and consequently cannot be termed as growth rings but merely as irregularities.

The tracheids are arranged in radial rows (fig. 3) and have a tendency to be rounded. In general the radial diameter of the wood cells is much the same, about 58μ , while the lateral diameter is variable, often not more than one-third of the radial diameter. The thickness of the tracheid walls is usually less than 12μ .

The rays are mostly uniseriate but may be biseriate in part (fig. 4), brought about by the lateral division of one or a few of the ray cells. The height is variable, ranging from 1–40 cells. The average is usually more than 10 cells but less than 20. These cells are about 23μ high, which is equal to the horizontal diameter. The length is quite variable, for they may extend over from $1\frac{1}{2}$ –7 cells, usually 3–4. There is considerable irregularity in the radial surface of these cells. The ends may be square, rounded, angular, or conspicuously contracted. This may be due to a partial decomposition of the original wood and pressure during petrification. In some instances the brown (resinous) content (fig. 2), which is so evident in many of the ray cells and in some of the tracheids, is distinctly contracted from the cell wall. The marginal ray cells are often twice as high as the regular ray cells. There are from 1–5 pits in the lateral walls of each ray cell and its adjoining tracheid (fig. 2). One and two are most common (2 is the number which seems to predominate), whereas the marginal ray cells may have 3, 4, or 5.

The full-bordered pits are distributed in 1 or 2 rows throughout the tracheids (fig. 1). Only a portion of 2 tracheids was observed in which there were 3 rows of pits, but these were considerably smaller and different in appearance from the ordinary ones. Ordinarily the pits measure $12\frac{1}{2}\mu$ in diameter with the lenticular orifice $\frac{1}{3}$ of that size. Rarely are the pits crowded to such an extent as to cause them to appear hexagonal.

As mentioned previously, the material here described appears to conform in some respects to Penhallow's (1900) description of *Dadoxylon prosseri*.

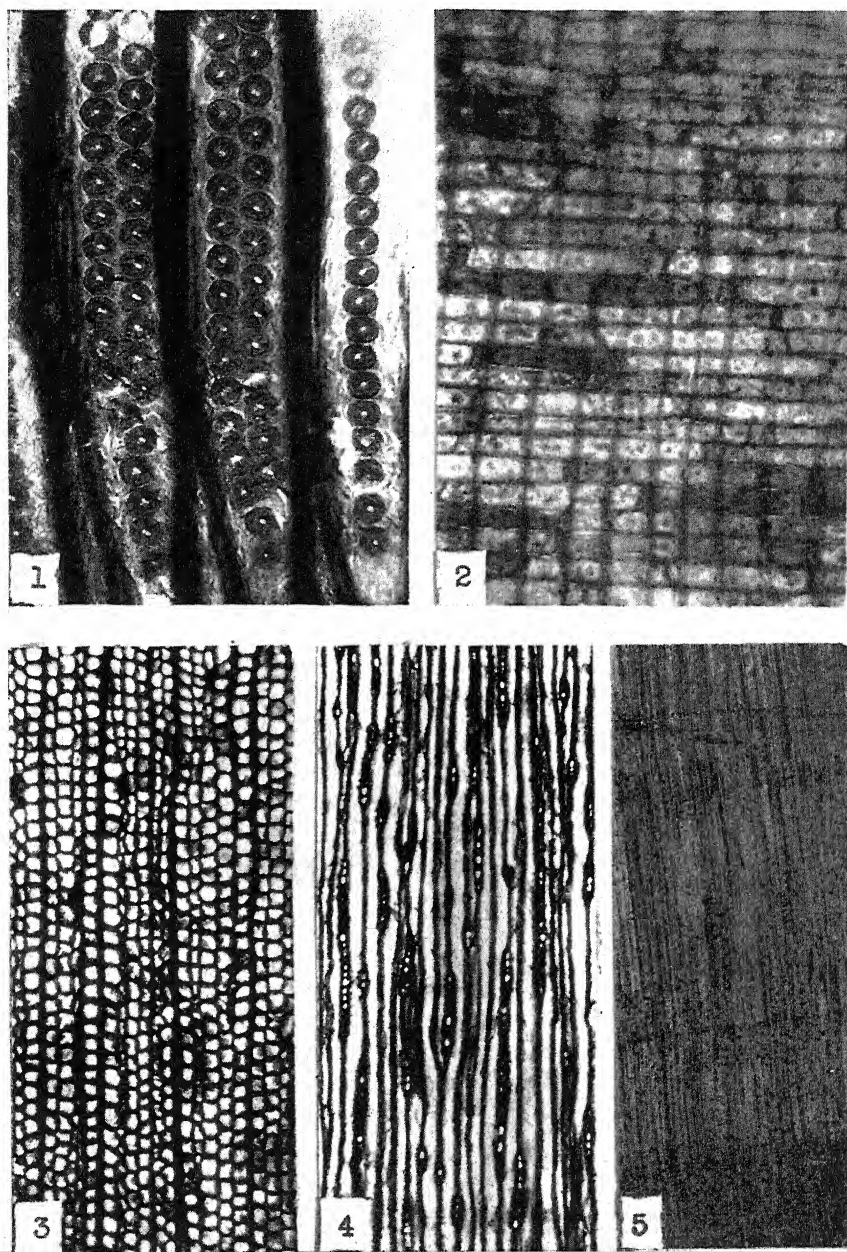


Fig. 1-5. Fig. 1. Radial section of *Dadoxylon douglasense* showing the uni- and biseriate arrangement of the pits. $\times 350$. Fig. 2. Radial section showing the lateral pitting of the ray cells and the resinous cell contents, often shrunk away from the cell wall. $\times 140$. Fig. 3. Transverse section of *Dadoxylon douglasense*. $\times 40$. Fig. 4. Tangential section showing the seriation and the variation in the height of the ray cells. $\times 44$. Fig. 5. Transverse section of *Cordaites materiarium* with growth rings. $\times 5$.

There are, however, several differences which distinguish the two to some extent. The height of the rays, the length of the ray cells, and their lateral pitting constitute the main differences. Penhallow does not give the height of the ray cells, but a study of his specimen would indicate that they are not as tall as those described here. The length of the ray cells of his specimen is described as extending over 2-4 tracheids, while those in the new material have a greater range than that. Penhallow also says that the lateral walls of the ray cells have from 2-4 pits. In the material described here, one pit is exceedingly common while four are rare and then confined mostly to the marginal ray cells. The slight difference in the seriation of the pits in the two rather swollen tracheids may be considered as an abnormality and of no important significance. There are also discrepancies in the sizes of the various types of cells, but these measurements are considered of little value because they are based on measurements obtained from a small fragment of a specimen.

Because of the differences in the height of the rays and the lateral pitting of the ray cells, and because of the fact that the material here described is in a better state of preservation, which makes it more valuable for further reference, it appears advisable to describe it as a new species. Likewise, the Douglas formation, in which *Dadoxylon douglasense* occurs, is separated from the lower Permian by the two upper formations of the Pennsylvanian, the Shawnee and the Wabauense. If the two occurred reasonably close together stratigraphically, it would be justifiable to disregard the original type of *D. prosseri* and to reestablish this species on a neotype. This course is recommended if new collections show that the type of wood here described really extends into the Permian, with enough variation to obscure the distinctions which have been pointed out.

Cordaïtes materiarium Dawson. The specimen from Coffeyville, Kansas, is a fragment of what was evidently a large trunk. The piece measures 15 cm. in length, 3 cm. in radial diameter, and $5\frac{1}{2}$ cm. in lateral diameter. All of the material is secondary wood from one radius of the stem.

Of all the material described by Penhallow (1900) and Dawson (1863), the Kansas material examined by the writer resembles *Cordaïtes materiarium* most. Several points of difference will be pointed out, but on the basis of secondary wood only, these do not justify the description of a new species.

A thin section of the material shows three distinct growth rings (fig. 5) and portions of two others. The "summer wood" is only 2-5 cells in width but distinct. The broadest of the rings measures $6\frac{1}{2}$ mm., the next 5 mm., and the narrowest $3\frac{1}{2}$ mm. Dawson (1863), in his description of *C. materiarium*, refers to the "rings of growth slightly marked," while Penhallow (1900), in his description of the same material, makes no reference to them at all. Goldring (1921) does not consider growth rings a characteristic of sufficient importance to separate or establish a species.

The distribution and the size of the bordered pits present another slight discrepancy. Penhallow describes the pitting as 2- to 4-seriate. In the material described here, one row of pits is comparatively common, three rows

are rare, and four rows have not as yet been observed. Holden (1917), Schacht (1861-1862), and von Mohl (1861-1862) in their work point out that irregularities may occur in pit arrangement and it may be inadvisable to use this as a diagnostic characteristic of secondary woods of which a limited amount of material is known. The pits of the later specimen range from 12-15 μ in diameter, and Penhallow describes those of *C. materiarium* as 12 μ .

The rays of both specimens are up to 40 cells high and generally uniseriate, and the cells are similar in size. They are also of the same length, extending over 2-6 tracheids. Penhallow describes his material as having 1-5 pits on the lateral wall of each ray cell and its adjoining tracheid, usually 1-2. In the later material the range has been found to be from 1-4 with 1-2 predominating.

Although there are dissimilarities between the two specimens, they are of such a nature that they may reasonably be accounted for as variations which are present in secondary wood or possibly in observations which arise from the varying preservation of the material. Because of this it appears advisable to refer it, at least for the present, to *Cordaites materiarium* Dn.

Probable horizon, Marmaton group, lower Pennsylvanian. Locality, Coffeyville, Potato Creek, Montgomery County, Kansas. Type no. 15113, Museum of Paleontology, University of Michigan.

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PERIGENESIS AND THE CONTROVERSY OVER PARASYNAPSIS AND TELOSYNAPSIS. I.

HAROLD C. SANDS

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The assumption of a telosynaptic union of chromosomes with suppressed or delayed abstriction would simplify most of the papers on parasynapsis which attempt to explain fragmentation, translocation, inversion, and recombination in various combinations.

Cleland and Blakeslee (1930) make the following statement: "That homologous ends are normally adjacent at pairing is shown in other forms: by chromosomes, the two ends of which are distinguishable by the presence of arms and by the location of chromomeres of different sizes as well as by other evidence. It is also the basis of the present conception of linkage and crossing-over. It is not yet known, however, in what the likeness of the ends consists which makes them attract. The hypothesis that such attraction brings together homologous parts of chromosomes seems amply justified by all the chromosomal phenomena in *Datura* so far analyzed." Blakeslee (1929) suggests a more detailed reason.

This paper is limited to the stages from the second contraction pachytene through the anaphases of the first division to emphasize these events before presenting the interkinetal, second division, and haploid figures.

Continuing my paper of 1925, the heavy pachytene contraction opens out in the form of either closed or open rings, rods, or a serial chain of elements which have arisen from a process of constriction and abstriction. Leliveld (1928) concluded the rings were formed by the accidental closing of the free ends as described by me in 1925. Originally this was my interpretation in *Tradescantia* and *Rhoeo*. However, conditions in *Oenothera* discussed later have led me to modify this view.

The linkages in these forms are the natural expression of a continuous spireme. A paper by Hoar (1932) practically repeats my statements as to a continuous spireme (1922-1925) in describing his observations on *Hypericum* and agrees in this with the conclusion of Gates (1908), Stout (1912), Cleland (1922-1931), and many others.

My 1925 paper defined the primary constriction as that which marks out tetrad linkages from a continuous spireme or the equivalent. Separation through this constriction leads to *primary abstriction*. The secondary constriction appears between bivalents, and segmentation through it leads to the *secondary abstriction*. The tertiary constriction marks out univalents, and separation through it leads to the *tertiary abstriction*.

Chambers and Sands (1923) separated the primary constriction of an octad ring but found no especial tenacity where the free ends joined. The secondary constriction joining two bivalents was more tenacious. The tertiary constriction was quite the most tenacious. This is shown by the relative amount of constriction the elements exhibited in figure 9, plate 1 (1923), when stretching was applied with the micro-dissection apparatus.

OBSERVATIONS

Plate 2 of this paper presents a series indicating the origin and development of the rings and rods. The stages are from material fixed with Flemming's medium solution and stained with iron haematoxylin. The sequences for the opening of the contraction figure (synizesis) would be figures 1, 2, 3, 4, 5, 6, 7, 8, and 9. In figure 7, three rings are seen coming into view. The fine strands of achromatic substance show the independence of the ends at this time. In figure 4, an earlier stage, only one ring is seen, whereas figure 8 shows four rings clearly. These are all from the same flower.

In figure 5 the constrictions demarking the elements of the thread are advancing to abstriction. Figure 6 shows a still more advanced stage of this process, while figure 28 (pl. 4) is a drawing from an aceto-carmin preparation. The point of interest is the achromatic bridges indicated by *a* and which no doubt would soon have parted. Plate 1 is an enlarged photograph of figure 9, plate 2, and demonstrates that these constriction fibers may be preserved in fixed material. In this case the fine achromatic bridge is unquestionably under tension.

Vom Rath (1891) was one of the first to see this ring formation. He says: "Die Fädenabschnitte verkürzen sich und die Schwesterfäden jedes Doppelsegmentes verlöthen an ihren freien Enden mit einander (fig. 13*d*) und so entstehen im Kerne sechs chromatin Ringe u. s. w."

Delayed abstrictions

Delay in the completion of the primary abstriction often brings to the metaphase spindle of *Tradescantia* linkages up to octads or more. The linkages shown in text figure 1, *A* and *B*, are quite rare. In this figure a closed chain occurs in *A*; an open one in *B*. A comparison with *F*, a tetrad, indicates that *A* consists of four tetrads in closed linkage. In *Rhoeo* as much as half or even all of the spireme in continuous linkage has been observed on the first division metaphase plate (Sands, 1925, p. 182). This has also been confirmed and figured by Kato (1930). In figure 29 (pl. 4), *Rhoeo*, a linkage of three tetrads is shown. The primary constrictions are at *a* and *b*, the secondaries at *c*. The tetrads are 1, 2, and 3. The tertiary constrictions are not yet evident. However, *Rhoeo* sometimes cuts off rods and rings as described for *Tradescantia*, and there would seem to be considerable plasticity in this, although Cleland's results with *Oenothera* hybrids indicate it to be a

specific character. Further delayed abstractions in *Tradescantia* are indicated in figure 22 (pl. 3). This continuous spireme, then, is clearly evident in several forms, notably *Carex* (Stout, 1912) and *Rhoeo* (Sands, 1925; Kato,

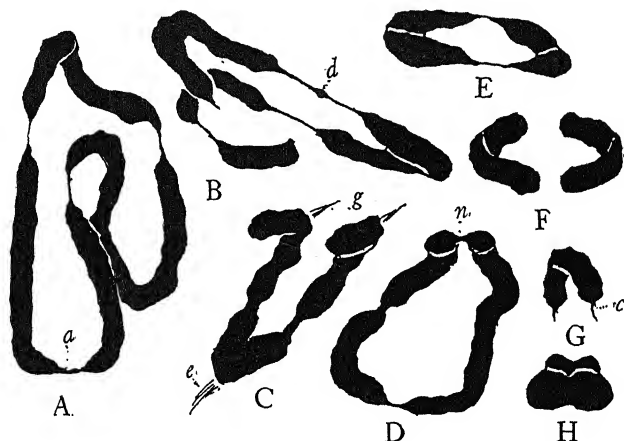


Fig. 1. *Tradescantia*. A, closed circle of 4 tetrads; B, open string of 3 tetrads with both primary and secondary constrictions present; possible chromatin elimination at (d); C, a hexad indicating fiber insertions; D, closed octad with secondary constriction not yet initiated in the right half; E, a closed tetrad ring; F, a later development of E; G, anaphase first division dyad with persistent ends of both primary and secondary abstractions; H, end view of dyad indicating double nature (cf. fig. 32, pl. 4).

1930), while many *Oenothera* workers figure it. It is discussed for *Hypericum* by Hoar (1932).

Octads

In *Tradescantia* a very common delayed primary abstraction results in an octad linkage which occurs in about 25 per cent of the cells. Cleland (1932) finds a similar linkage in *Oe. franciscana* which is practically always present, while Kulkarni (1929c) finds it in hybrids of *Oe. pratincola*. Digby (1912) reports one in *Primula*.

This octad forms various elements of closed or open rings during the metaphase. Both primary and secondary abstractions may proceed simultaneously although not always. In figure 10 (pl. 2) and figure 16 (pl. 3) the octad is seen as a closed ring. In figure 12 (pl. 2) the ring is opening, and a thread-like strand of fine achromatic substance still persists between the separating ends. In figure 17 (pl. 3), an aceto-carmin preparation, the element lies on its side with the ends free. (See similar figure and discussion, Sands, 1925, figure 8, pl. 1).

On plate 4, all drawings of which are from aceto-carmin preparations, figure 25 shows this linked group on the metaphase spindle overlying the other elements. The primary constriction is at a. In figure 27, the element overlies other elements of the complex while the polar distribution is indi-

cated. In figure 5, plate xvii, of Shinke's (1930) photographs of *Tradescantia*, a linkage of three tetrads is apparent, whereas the octad element mentioned is directly adjacent to the left. The extreme right element would

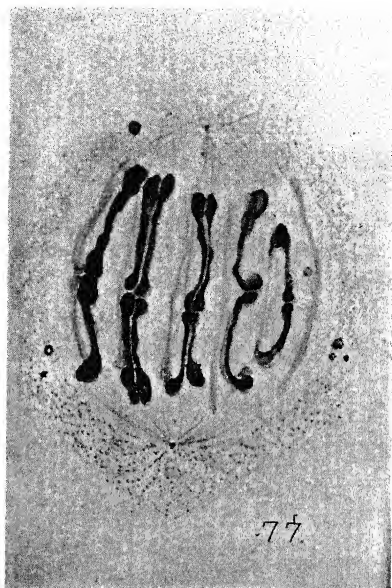


Fig. 2. Transverse and longitudinal separation as conceived by Farmer and Moore (1905). The longitudinal split is an optical artifact resulting from an achromatic medulla. Microdissection fails to demonstrate it as otherwise. (From 2. J. M. S. 48; pl. 40, fig. 77.)

be an open arrangement of the four-tetrad group *A*, text figure 3 of this paper.

The locus of the fiber insertion is spread over a surface rather than limited to a minute point. The base of the fiber in contact with the element is the

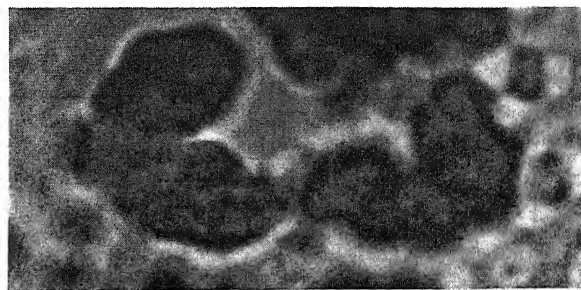


Fig. 3. Key figure demonstrating the origin of the first division spindle anaphase dyads by a process of constriction and folding. Enlargement from the negative of fig. 14, pl. 3 (Original).

base of a cone shown well in figure 19 (pl. 3). In most cases the anaphases present V's (dyads) with equal arms (isobrachial); where foreshortening occurs, they appear unequal.

Figure 34 (pl. 4) is a metaphase developed from a diakinetik arrangement, and the 12 tetrads are numbered 1, 2, 3, etc., for identification. The beginning of the tertiary constriction is to be noted at *b* in tetrads 1, 2, 4, and 8. The point *b* in 8 is a stage just prior to *b* in element 15, figure 26, and is followed immediately by the development shown in element 16 of the same figure which now effectively marks out the anaphase dyad. Interlocking rings as described by Cleland (1922) have not been observed. The octad ring, then, in figure 10 (pl. 2) arose, no doubt, from a form like that seen in figure 17 (pl. 3) by the association of free ends.

Hexad rings and linkages

A hexad ring, comparatively few of which are seen in either *Tradescantia* or *Rhoeo*, appears to be developing in figure 28 (pl. 4). (Cf. text figure 1, C.) Such a figure is apparently illustrated by Bleier (1931), who figures it also on page 64, text figure 12, of his paper. I interpret his diagram to be a linkage of three tetrads (in my sense). A hexad (trisome of Belling and Blakeslee) would be a linkage between a tetrad and a bivalent where perhaps the bivalent represents a supernumerary. A hexad structure appears in Webber's (1932) paper in his text figure 3, *B* and *C*. The tertiary constriction is indicated in his drawing.

A somewhat analogous linkage may be found in the *XY* components of *Tenodera* (Oguma, 1921; cf. also Wilson, 1925, fig. 373) where the linkage, as a result of perigenesis, would be (*XX-YY-XX*) with the tertiary constriction still undifferentiated. Half of the sperms would have a *Y* while the other half would have *XX*'s.

In general, supernumerary univalents of most authors, but looked upon as bivalents by me, usually abstrict early and either separate on the first spindle as true univalents or go over whole as an anaphase dyad to separate on the second. They may be eliminated entirely from the nucleus (Juel, 1897). Belling and Blakeslee (1923) figure closed ring hexads in their triploid *Daturas*, their so-called secondaries.

In triploids, hexad linkages may or may not occur according to the compatibilities, positive or negative, at work. Rosenberg (1917), in *Drosera* crosses, did not note any qualitative likeness in the supernumeraries causing associations, whereas Steere (1932) figures perfect hexads in the trivalents of his figure 2, plate xxiv. Steere further discusses the intergrades up to auto-triploids—i.e., all tetrads linked with a bivalent leaving no independent rods. Steere further groups his triploids into classes on the basis of maintenance other than by apomixis.

Cleland (1931) suggests that the more heterozygous the individual, the greater the attraction of the free ends to form extensive linkages. This un-

likeness of ends is more freely discussed in his paper of 1926 as well as by Blakeslee's paper of 1929. (Cf. also Belling and Blakeslee, 1926b.) Some of the behavior would indicate that the individual chromosomes of the same complex may have varying degrees of compatibility or incompatibility with respect to associating ends when brought into relation with the homologues of another individual. The phenomena may even be allied to the expressions of gynogenesis.

In these cases we find many authors discussing end-to-end relationships but rejecting the idea of telosynapsis in order to support crossing-over. Steere's supernumeraries in autotriploid forms as well as tetrads are joined *end-to-end*. The discussion here does not treat of sides. These forms in *Petunia* show one of two things, either delayed abstriction at specific points or they are the result of end-to-end conjugation. According to Blakeslee and Cleland's hypothesis (1930), Steere's autotriploids do not form a closed trisome (hexad in my sense) because the outside end of the supernumerary bivalent is not homozygous with the other free end and is therefore incompatible. The same end-to-end connection occurs between the XX element in *Drosophila* (Morgan, 1922). Since the XXX forms die, the result may not be compared to these shown in Steere's figures.

In view of the complete continuous linkages in the forms already noted, it would seem more logical to consider the connection of the supernumerary in these hexads to be due to delayed abstriction rather than to independent conjugation. It may be looked upon as an expression of plasticity in the process of meiosis. Where the supernumerary is completely separated (early diakinesis), the effective agent would be the primary abstriction acting on one end with the secondary on the other, since the allelomorph is absent.

Tetrads and tetrad rings

Tetrad rings arise early from the contraction forms presented by figures 4, 5, 6, 7, and 8 (pl. 2). They cannot be confused with octads, since in figure 10 both forms are photographed. In ring tetrads the free ends come together from the flexing habit of the segments as described for octads and hexads. Cleland believes this to be specific as noted. The achromatic bridges are well shown in figures 4 and 7 (pl. 2) and indicate that this stage is the beginning of ring formation. I assume the rods to be essentially the same elements as the half rings except that the flexing habit is less developed.

The separation of all members of the complex on the spindle is not always synchronous, so that it cannot always be told whether a given rod has passed through a closed or open ring form. Figures 26 and 34 (pl. 4) lead one to suspect that the ring may be quite common. The cross-shaped tetrads described for some animals and plants have not been noted in either *Tradescantia* or *Rhoeo*. Edge views of rings appear like transversely separating bivalents, and vary in length according to the degree that the half ring plane-projection approaches the rod length as a limit. I consider the second and

third elements from the left in text figure 2 to be edge views of partially opened rings. The outside arms are foreshortened.

The manner of separation of the tetrads is well illustrated in figure 26 (pl. 4), as already discussed in the last paragraph under octads. This figure is a



Fig. 4. Belling's haploid *Datura* chromosomes constricted in the middle indicating the presence of sister identities as a result of perigenesis. The tertiary constriction here further supports the assertions of my paper (1922). (La Cellule 37, fig. 3.)

drawing of the photograph in figure 14 (pl. 3) labeled for reference and discussion. Elements 7 and 8, figure 26, would be comparable with the left elements in photographs 20 and 21 (pl. 3). A progressive separation series from figure 26 would be elements 13 and 14, 19 and 20, 9 and 10, 1 and 2, 15 and 16. A still further state of separation, but maintaining the achromatic bridge, is seen on the right of figure 24 (pl. 3). This fine strand often persists into the late anaphases. Usually it is the secondary constriction and it forms the traction fibers of the older authors. Figure 21 is an intermediate stage between figures 19 and 24. In figure 20 the spindle attachments are seen to be quite stout. The secondary constriction seems to offer resistance, and it would appear that the fibers were doing work on the joint. At least they do not appear to be lines of flowage (cf. Bleier, 1931, on Abstosskräfte). The force causing constrictions is, however, independent even though at times the fibers seem to assist in abstriction. More evidence for this will be given in the second division data.

The distinction between my half rings, two chromatids and therefore bivalent, and the bivalents of Haecker (1892), Mottier (1903), Farmer and Moore (1903, 1905), and many others is to be noted. Farmer and Moore would consider my elements 21 and 22 (fig. 26, pl. 4) and both 6 and 7 (fig. 34, pl. 4) as bivalents. As remarked by me in 1925, they require only the constrictions of *Arisaema* (Atkinson, 1899), *Pteris* (Calkins, 1897), and *Chiloscyphus* (Florin, 1918) to make morphological tetrads of them. In other words, the *tertiary constriction* has appeared earlier in those forms than usual. Thus there have arisen the *Querkerbe* of Haecker (1895) and the sutures of Marcus (1906), Kornhauser (1915), and others.

It is now more clearly apparent why I consider Blakeslee's trisome to be a hexad. In his publication with Cleland (1930), the numerical diagram at the foot of page 178 is clearly of an octad condition, while at the same time the text discusses it as a tetrad. No allowance has been made for *perigenesis*.

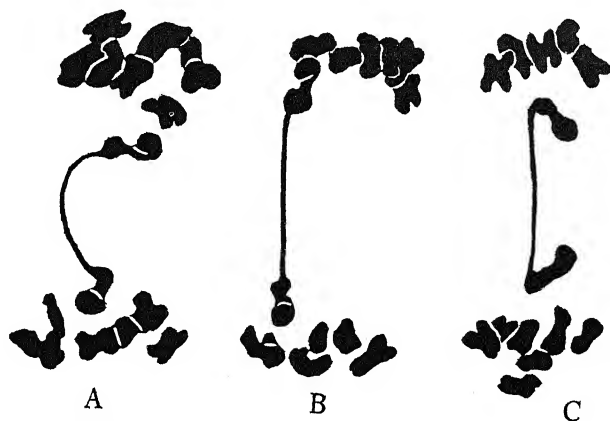


Fig. 5. Three modifications of a tenacious secondary constriction. The four chromatids in each case are clearly demarked. The figures are a logical development from configurations like figure 2. *A*, high arch; *B*, tension bridge, chromatids in straight alignment; *C*, tension bridge, chromatids in reversed alignment.

Let us return now to a consideration of elements 15 and 16 (fig. 26, pl. 4). (See photograph, fig. 14, pl. 3.) I have enlarged this as text figure 5, because I consider it a key figure that will explain much of the confusion explained as longitudinal splits, especially by Farmer and Moore (1905). We have to do here with a folding at the suture of the tertiary constriction as I have outlined it. This develops the dyad indicated by the number 16 but not yet separated from the other half ring labeled number 15. The secondary constriction here has apparently lagged beyond that shown between 5 and 6, 3 and 4, or 17 and 18. Elements 9 and 10 are well on the way toward duplicating the form of 15 and 16, whereas 23 and 24 have apparently separated early and before the tertiary constriction is far advanced. A consideration of polar crowns as well as late anaphases shows, as indicated by figures 30 and 32, that the tertiary constriction eventually sets in and the folding of element 16 (fig. 26) is finally arrived at. The dyad developed in this manner is morphologically indistinguishable from those illustrated as having arisen from a split. The anaphase dyads of the first reduction division arise by a process of *constriction and folding*. The tertiary constriction, moreover, is persistent through interkinesis and accounts for the interkinetic doublings of Cleland (1922), Kulkarni (1929c), and others.

A progressive series of ring openings, using fixed material, would be: the left element, figure 20; the left element, figure 23; the left element, figure 19; and the right element, figure 24. Kaufmann (1926) notes this 4-parted

structure in the metaphases of first meiotic division in *Tradescantia pilosa*. Apparently he had not seen my 1925 publication; but later, in describing the chromosome morphology in *Podophyllum* (1929), he figures a ring tetrad and refers to it as such. The second cross-suture he calls the secondary constriction in contrast to my tertiary constriction. By this, he leaves no designation for the constriction which, by abstriction, developed the tetrad from the spireme and which I defined as the primary abstriction. These tetrads are not to be compared with false tetrads caused by the recurved associated arms of dyads with all four ends in the same focal plane. Such are especially common in the polar anaphase coronas of the first division as well as in the homeotypic division.

Chromatids

Returning again to figure 26 (pl. 4), the chromatids are indicated in elements 15 and 16 as *a*, *b*, *c*, and *d*. The enlargement (text fig. 3), as noted, shows the details intimately. The separation occurs rapidly and generally is missed for the reason that the secondary abstriction may proceed quite independently of the fibers. The formation would be observed then in those cases where this abstriction lagged or the joint for some reason was unusually tenacious. The chromatic particles have already been aggregated to *a* and *b* with but a colorless achromatic bridge connecting them (cf. Vom Rath, 1891), as quoted p. 403. Vom Rath later injects the idea of a longitudinal splitting of the bouquet spireme (Roux, 1883) and so misses my observations. All four units were therefore present in the contraction figure, and the pollen mother cell was tetraploid but not in the sense that Belling and others have used the term—i.e., originally double diploid. At diakinesis of such, octoploidy would exist.

Rückert (1893), working on *Heterocope*, describes the separation of the rings and tetrads he found. He says: "A ring is thus formed and tetrads arise later by two divisions, one through the diameter of the original cleavage, the other at right angles to this line." Since he considered the open spireme to be double, he was forced to consider the separation along the diameter of the ring as the homologue of the longitudinal split; otherwise he might have proposed two transverse abstrictions and one constriction, which would have been more in harmony with the predications of Weismann, who called for transverse separation.

Although Atkinson (1899), Gates (1908), Stout (1912), Kihara (1919), and more especially late *Oenothera* workers have found segmentations of a continuous spireme, none has called attention to nor has differentiated the apparent split that so many authors cite, from optical effects caused by the achromatic medulla of the filament. If a split were present, then with Chambers' dissection apparatus (1923) the elements ought to be separated along this split, assuming adhesion were not too strong. This cannot be accomplished even as late as the metaphases where the fission is presumed to be completed. The elements behave everywhere like *homogenous cylinders*.

Kaufmann (1926) figures the cylindrical cross-sections in the earliest prophases (cf. his figure 39, plate vii). He then proceeds with his spirals and must split them in turn again to make events harmonize. (See also Sakamura, 1929; Kuwada, 1927; Taylor, 1931.)

Returning now to this paper, figure 18 (pl. 3), the photograph is of the late anaphase of the first division. Figure 33 (pl. 4) is drawn from the same cell. Note the plump round ends preserved by the 10 per cent cane sugar or aceto-carmin suspensions but often shrunken and fused by strong coagulating chemicals. This relation of the dyads is clearly evident in the anaphase polar crown (fig. 30). In the element 4 of this figure, the achromatic bridge (tertiary constriction) seems to have slipped around to a point back of the ends. Some of the others show a side end movement with a relatively wide space between the elements. Davis (1911) and Cleland (1926) figure *X* forms in the first spindle telophases or crowns. This is caused by a further shifting back of the bridge insertion. The original bridge connections developed by the point of tertiary constriction may readily shift at this stage. It is not rigidly fixed but it is stable and persistently binds the two chromatids. Thus arise the sharp *X* figures of Sussenguth (1921) and Kato (1930, fig. 22a, pl. xiv).

Neither Cleland (1922) nor Kulkarni (1929c) reports checking fixed material against the living for this stage. Unquestionably they are interpreting as a univalent a fused dyad as figured in my text figure 1 (1925). Where reagents fuse such gross forms, not much can be hoped for such a delicate structure as the chromomere relationship within the chromosome. Where it can be determined, emphasis should be more on conditions before fixation. In most preparations not even a stamen hair is left.

In my figure 30 (pl. 4) of aceto-carmin material, the chromatids are widely separated. The twelve pairs can be counted. The cell is still tetraploid, as it has been from the previous resting condition where perigenesis occurred. It remains tetraploid till the formation of the cell wall that will separate the two hemispheres, each of which then becomes diploid. A quantitative reduction has brought conditions back to the somatic numbers while simultaneously segregation has occurred.

Examine for a moment Cleland's (1922) figure 28, plate xxvii. There is present an octad ring and four tetrads, a total of 28 univalents. The same holds for his figures 25 and 26 of the same plate, making these cells tetraploid at this stage and not diploid as in the usual sense. The chromatids may exist, therefore, directly adjacent to each other united end-to-end but momentarily undifferentiated as such, considering Belling's haploid *Datura* morphologies. In text figure 4 they are differentiated at an early stage. The median constriction here marks them out and further supports the doctrine of perigenesis. The cell, while looked upon as a haploid on account of the absence of allelomorphs, is really quantitatively diploid. The constriction could be submedian if segmental interchange should occur in the haploid of a heteromorphic complex or if the units were unequally hydrolized.

It is probable that Hutchinson (1915), Chamberlain (1916), and Weniger (1918), in spite of reports against transverse segmentation, came very close to conditions being developed by me; and of the three, Weniger's account seems the closer. However, I shall develop this further at another time.

As Sharp (1921) points out in his text, p. 298, Hutchinson's interpretation of transverse division involves a fragmentation. No allowance, however, has been made for perigenesis (synthesis of matter) in the haploid gametes, either prior to or during the fertilization process and before syngamy, or following syngamy prior to the first cleavage. Here also it is not necessary that the two sister chromosomes be at once morphologically apparent. The gross mass outline may not reflect the internal organization of the chromatin which obviously is also a matter bearing on the compound nature of some chromosomal bodies. That Hutchinson's complex was tetraploid prior to telophase I am convinced is correct, as I am pointing out for other cases.

DISCUSSION

The significance of reduction versus equation in the accepted sense does not apply so strictly in this state of affairs except as pointed out by me in 1925—i.e., the transverse separation of elements from end-to-end relationships. Goldschmidt (1932), citing Dodge's (1927) findings on *Neurospora*, says: "The problem for the necessity of the equation division disappears if, for individual chromosomes, both maturation divisions may be reductional and that therefore there is no equation division but instead two supplementary \pm reductional divisions."

The terms anaschistic and diaschistic do not apply at all. If the second division spireme (where formed) abstracted dyad linked-elements from end-to-end relationships and these be sisters, then the conception of an equation division would apply. Reduction would apply to the separation of allelomorphs on either spindle. The numerical reduction of cytosome chromatin in these two plants, however, does not occur till the completion of the tetraspore walls or furrows. Reduction might be homologized with synapsis; however, I look upon synapsis to be more closely associated with nuclear reorganization and the disassociations following perigenesis. On the other hand, I am not excluding rejugation, which sometimes follows, as will be shown later from *Rhoco* material. To what extent reduction occurs on the first versus the second spindle can only be studied cytologically in a heteromorphic complex or else in XY combinations. Even with heteromorphism, separation might be closely restricted to homozygous units. It would require segmental interchange between unequal chromosomes to give a workable form for observation.

The character of the first division as to reduction is bound up with the relative position of paternal and maternal elements in the continuous spireme—i.e., in their alternations. It is the points to which the primary, secondary, and tertiary constrictions are applied which will determine the character of the tetrad at diakinesis. The possible variations of this sequence are outlined

by Blakeslee (1929) in his discussion of primaries, secondaries, and tertiaries. By my conception, in one instance a chromatid may be abstricted by a primary and tertiary abstriction; but if it became a supernumerary in another complex or were a part to segmental interchange, a secondary and tertiary abstriction might separate it. The events which transpire on the first metaphase spindle would be preordained in the disassociations and streamings of the previous resting stage.

In the doctrine of perigenesis, therefore, equation division could apply to two events, the transverse separation of identical chromosomes or the separation of like materials formed by a process of synthesis.

If the chromatin in *Achromatium* divides as figured by Schewiakoff (1888) prior to cytosome division, perigenesis as I have suggested it—viz., a respiration phenomenon leading to synthesis, and forming duplicate entities while approximately in a state of rest—is an example which is self-evident, even though the particles do divide amitotically.

Parasynapsis

No parasynapsis was found by me not explainable by a false longitudinal split due to the achromatic medulla of the earliest filaments and later stages or to the random parallel juxtaposition of the threads themselves. The same holds for the reports of *Oenothera* workers besides those using many other forms.

The controversy is of long standing; but as pointed out by Gates (1924), its retention for *Oenothera* demands that the doctrine of the individuality of the chromosome be abandoned on account of the resulting fragmentation. The same is true for *Tradescantia* and *Rhoco*. The morphologies figured on the accompanying plates are too gross to be misinterpreted. As a result of the type of fragmentation that text figure 3 would cause, if this were composed of two longitudinally appressed segments, no viable pollen would be expected. If rings originate only from parasynaptic inversion-conformations, as suggested by McClintock (1930-1931), then are we to assume multiple inversion in all the elements of a complex like that of *Oe. strigosa* where all chromosomal diakinetik forms are expressed as rings? Or take figures 26 and 34 (pl. 4) of this paper. In order to avoid the fatal results from such a fragmentation, parasynapsis needs a complex accessory hypothesis leading up to diakinesis.

It is understandable that either disjunction or fragmentation followed by telo-reconjugation would explain the attachment of pieces to other non-homologous chromosomes if one modified the idea that only similar or identical ends may associate. This does not explain the inclusion within the body of a chromosome, of foreign segments whether inverted or not. McClintock's (1931) paper has been widely acclaimed as explaining this. For many forms a satisfactory harmony of the sequences does not exist in view of the observations reported in this paper, and this applies to other papers reporting

continuous spiremes. The development, from paired elements, of homogenous chromosomal cylinders with an achromatic cortex not necessarily confined to the grosser late stages is illogical. The origin of rings from inversion is only in very small part satisfactory, since there are much too many rings; the existence of chiasmatype twists in gametophytes is ignored because the allelomorphs are presumably absent. Some deletions have a way of reappearing in the progeny, so that only deletions due to a true deficiency would be expected not to reappear. The persistence, growth, and viability of deficient complexes must be extremely limited. Boveri (1907) and many followers have shown that loss of chromatin, except within narrow limits, is lethal.

Whatever be the conviction of others as to parasynapsis, my own conceptions lean more strongly toward telosynapsis as suggested by Farmer and Moore (1903) pending security in the knowledge that identical figures for chiasmata and pairings in gametophytes have no significance, that deletions (caused by actual deficiency in x-rayed material) never reappear in the progeny, and that the phenomena of synapsis, translocation, or inversion never occur *during the reorganization or reassembly of duplicated material* immediately following growth where it could occur (cf. Fick, 1907); also, that an acceptable accessory hypothesis be found to explain the continuous spiremes discussed and so harmonize parasynapsis with the abstractions of the subsequent stages when longitudinal division does not exist here.

If parasynapsis be followed by a reseparation of elements to form diakinetid figures, the first division should be reductional (Grégoire, 1910) and not pre- or postreduction (Dodge, 1927; Goldschmidt, 1932). If parasynapsis occurred in haploids, one refuge would be that the haploid was polyploid or the chromosomes were compound; but parasynapsis between sister chromosomes is untenable, and crossing-over in haploid parthenogenetic forms would not be expected. Meves (1908) called attention to the pairings in the haploid vegetative stages.

It is generally conceded that the period from synizesis up to diakinesis or even the first metaphase plate is not a *period of growth* or duplication of materials. In many forms the tetrads are morphologically evident in stages leading up to and including diakinesis. The sister chromosomes are present, probably mirror images for the sister elements, and attached end-to-end. The sister chromosomes, therefore, should be present at the parasynaptic stage. So far they have not been described here. The possibility of their presence has not been considered in spite of the fact that diakinesis is nearby. In such an event, parasynaptic figures and morphologies should account for four similar architectures in these threads even to the so-called pynotic chromomeres. Whereas longitudinal splitting of the post-synaptic pairs coupled with reseparation of the parasynaptic originals has been figured in all sorts of complex configurations (cf. Wilson and Morgan, 1920) to explain the origin and separation of the tetrads of the first division, it certainly is not applicable to the plants I have dwelt on.

Perigenesis during interkinesis and haploid behavior

Where a complete state of rest occurs during interkinesis (if it does), the question arises: Are the two univalent chromatids described as entering the telophase the same individuals that emerge to be separated on the homeotypic spindle? This reopens again the argument as to the individuality of the chromosomes. I assume that perigenesis is suppressed during interkinesis, since this is not a growth stage.

Data from the haploid honeybee and hornet would seem to indicate, because of a homeotypic division, that interkinetic doubling had occurred. The chromosomes of these forms must come to the first spindle in diploid quantity by reason of perigenesis, as suggested for Belling's haploid *Datura*, although morphologically they may not be so differentiated. It would be expected they would either go over whole on the first division and be separated on the second (*Datura*, Belling and Blakeslee, 1923); separate on the first (Kihara, 1919) and go over whole on the second; or else part of the complex may follow the first process and part the other (haploid tomato).

Gaines and Aase (1926) report some end-to-end pairing of elements in a haploid wheat which can be followed from the late prophases but without the formation of rings. If in the prior resting stage there had been a duplication of material, then the three possibilities could develop as discussed in the previous paragraph. A linkage of two elements here would be potentially a tetrad. If, maintaining this linkage, they separated on the first spindle, as do the rings or rods of *Tradescantia*, the sister interkinetic nuclei would show double deficiency and double duplication. If they should separate through points homologous to the *tertiary constriction*, each nucleus would receive normal chromosomes. However, the permanency of this union up to the metaphase has not been described.

A partially continuous spireme is more definitely developed in the haploid tomato of Lindstrom and Koos (1931). Here, on account of the extent of the linkages, unlike ends must have associated or synapsed. Assuming perigenesis, there would be present as chromatids $\overline{1,1}$; $\overline{2,2}$; $\overline{3,3}$; etc. If segmental interchange occurred between the first two elements, then the formula would be $\overline{1,2}$; $\overline{1,2}$; $\overline{3,3}$; etc. By the association of like ends a potential tetrad would arise in two forms, either 1-2-2-1 or 2-1-1-2. The element would probably be in the shape of a bivalent with either open or appressed arms. However, the linkage is more extensive than this. Assuming only the association of like ends, then for a linkage of three, a double segmental interchange must occur giving units organized as 1-2; 1-3; 2-3; etc., which on rewriting would be $\overline{3-1} + \overline{1-2} + \overline{2-3}$, etc., a linkage of three bivalents. The chromatids may or may not be differentiated.

If one of the *independent* rods (bivalent by perigenesis) went over whole, one interkinetic nucleus would show a single duplication, the other a single deficiency. Diakinesis and interkinesis are both omitted, so that there is no

opportunity here for an interkinetal perigenesis. For those few figures in which all the elements went over whole, the case is exactly analogous to the bee, and the empty sister hemisphere is atrophied.

The important point, however, is that univalents as defined in this paper would not be expected to divide on both spindles. In most cases where supernumeraries are present they do not. In those cases where contradictions exist, notably in *Pygaera* (Federley, 1912), *Hieracium* (Rosenberg, 1917), and *Rosa* (Täckholm, 1920, 1922), comparisons should be made with the work of Kostoff (1930, 1931) and Kendall (1931), who report aberrant gametes, non-disjunction, and polyploidy often with the omission of the first division. In such back crosses, if the functional gamete arose from the suppression of one division, the individual would be triploid. In both the haploid tomato and wheat polar crowns part of the complexes have gone over whole, while other units have separated sister identities on the first division. The authors do not describe division of the same elements on both spindles.

Other considerations

Much of the confusion as to what occurs from the prophases onward may be traced to Roux (1883), who was one of the first to describe a longitudinal split. Most followers attempted to account for it either in the very earliest stages (leptotene), early spireme, synizesis, open spireme, metaphase, anaphase, telophase, or interkinesis with both single, double and finally, quadripartite spirals, the precocious splits of a future mitosis.

Let us return momentarily to the system advanced by Rückert (1893) and further developed by Farmer and Moore (1903-1905). These authors contend that the loops break apart from one another by a segmentation of the spireme. Thus far there is no objection. They state further that each element is composed of two split chromosomes arranged end-to-end. This is the point of departure, as already noted. No allowance has been made for the fact that the achromatic medulla, in plane projection, may look like a split when the structure is really something else.

Text figure 2 is from Farmer and Moore (1905, fig. 77) and represents what happens to their split *bivalents* on the metaphase spindle. The split figured is the optical effect caused by the peripheral arrangement of chromatin particles within a linen cylinder and shows the effect of severe action by fixing reagents. The anaphase V's are formed by a progressive furrowing along the line of the presumed split to stop at the fiber insertion while the arms open out. The formation of those arms by tertiary constriction, as shown by my figure 26 (plate 4) and photographed in text figure 3, was entirely overlooked. They would define the points of my secondary constriction as the points of original telosynaptic conjugation.

Their tetrad then consists of two contiguous masses, each longitudinally divided, which is an entirely different tetrad from that described by me. For the double V's entering the telophases, they have again figured the medulla of the folded chromatids. This has also led other authors astray.

Diakinesis in *Tradescantia* is merely a matter of priority with respect to the onset of the spindle fibers and the completion of primary abstriction. My figure 34 (pl. 4) would seem to be derived from a diakinetik expression, although the elements, in some cases, are clearly in the metaphase as well as in the anaphase initials. Where the primary abstriction is completed early, a diakinetik figure would be expected. In *Oenothera* a series of intergrades occurs, even to the omission of diakinesis. This seems to be associated as a specific character. If more genetic species of *Tradescantia* and *Rhoeo* were synthesized, possibly a similar series for each plant would come to light.

We have now seen several modifications of meiosis. In the case of the honeybee all elements go to one pole, the cytosome pinches off an empty cell, and a second division follows. In the case of the haploid tomato sometimes all chromosomes go to one pole, there follows a second division, the empty half degenerates, or else each half may get any combination down to one. In the megaspore or in animal ova, one haploid group functions and two polocytes (three haploids) degenerate or two function, as in the bear, or all four, as in the armadillo. In Phylloxerans and aphids half the sperm possibilities do not develop. In *Hieracium laevigatum* the homeotypic division is suppressed, while in *Hieracium pseudo-illyricum* the heterotypic division is suppressed. In the haploid tomato, since interkinesis is suppressed, perigenesis must be suppressed, and this seems logical from events in *Oncopeltis*. A definite plasticity underlies the whole process which is further emphasized by the behavior of inversions, translocations, fragmentations, and rejugations.

Some of the error in the observations on these gross morphologies has been recently called to mind by Jeffery (1933) and replied to by Belling (1933). The tetraploidy suggested by Belling for *Tradescantia* here is not the tetraploidy defined by me (1922) as resulting from perigenesis. Belling still failed to see the bivalent nature of the 24 undifferentiated masses making up the 12 rings. If the plant were a tetraploid, as tetraploidy is usually defined, then at diakinesis it would be octaploid with the equivalent of 24 rings present whether expressed as rings, rods, or linkages. He says further, in regard to *Rhoeo*, "Thus the 12 bodies seen in the rings appear to me to be these univalents attached at the ends." The same error occurs here. The haploid of *Rhoeo* is 6, and there is the equivalent of 6 tetrad rings present at the stage corresponding to diakinesis whether or not the elements are expressed as rings or a continuous linkage. My figures 26 and 34 (pl. 4) make these points clear. Part II of this paper will shortly follow.

SUMMARY

1. Meiosis in *Tradescantia virginiana* L. and *Rhoeo discolor* Hance develops elements from a continuous spireme. *Rhoeo* may bring all of the unsegmented spireme on the first metaphase spindle.
2. The first division anaphase dyads are formed by a process of constriction and folding and not by longitudinal splitting with subsequent separation of the halves.

3. No parasynapsis can be observed in either plant.
4. Apparent longitudinal splits are artifacts due to interpreting the plane projection of an achromatic medulla as a split.
5. The division of chromatic elements is a matter of transverse separation from end-to-end associations.
6. The creation of like materials is a process of perigenesis (Haeckel) followed by reassortment of the duplicated matter and not the converse—i.e., not the mechanical cleavage of an original particle.
7. Segmental interchange in haploids could best occur if there had been perigenesis as a result of growth in some prior stage or else as a process of fragmentation and re conjugation. The latter would not normally be expected. If like or homozygous ends only associate, then a continuous linkage of more than two haploid rods would seem improbable. The fact that more than two linkages occur in the haploid tomato indicates that some other condition than the association of only like ends must also be involved in these unions if multiple segmental interchange be not a factor.
8. The process of meiosis might be roughly likened to an amitotic separation of thread segments at certain specific points.

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WOOD RIVER JUNCTION,
RHODE ISLAND

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EXPLANATION OF PLATES

PLATE 1

A photographic enlargement of the primary constriction from the negative of figure 9, plate 2. Although fixed with Flemming's medium fluid, the fine achromatic bridge is well preserved.

PLATE 2

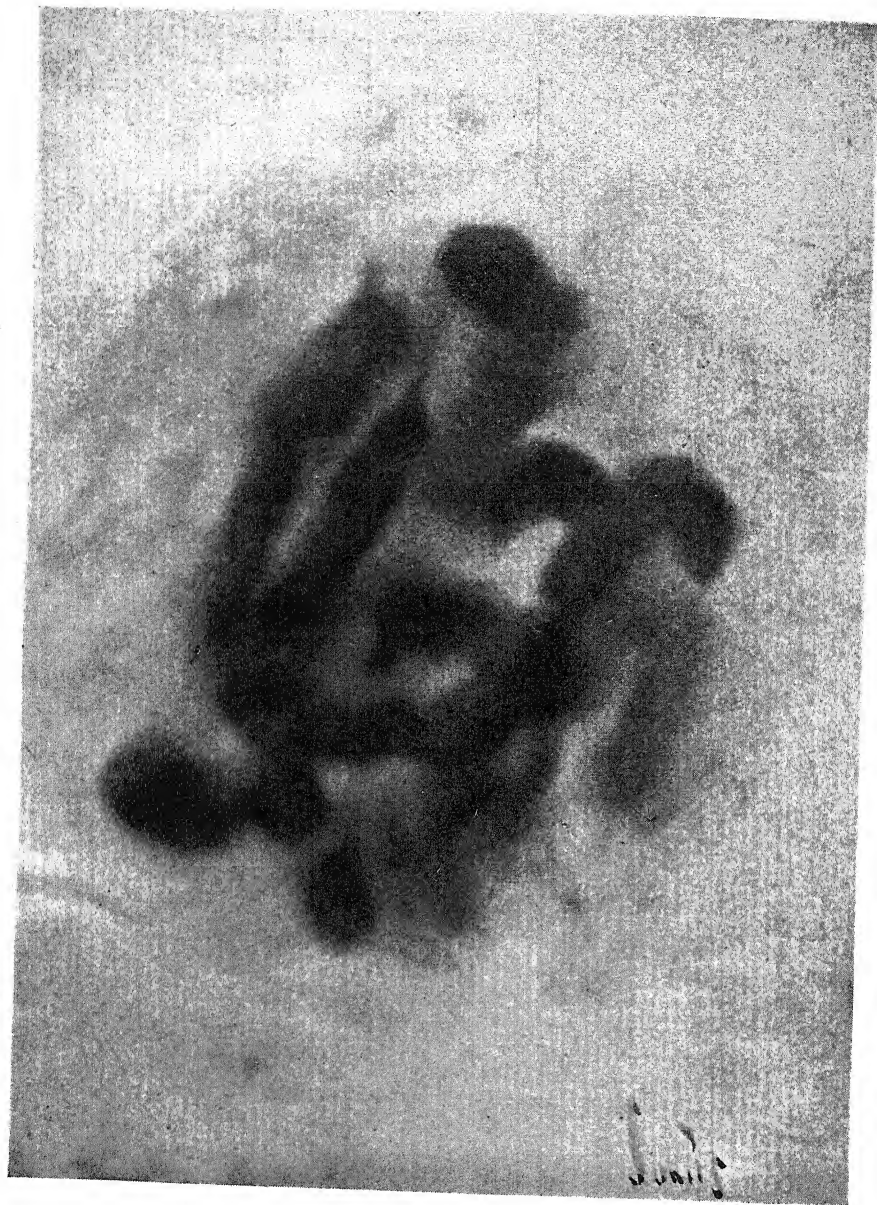
Material fixed with Flemming's medium fluid and stained with iron haematoxylin. All figures are from *Tradescantia* except figure 13, which is from *Rhoeo*.

- Fig. 1. Synizesis in *Tradescantia*.
- Fig. 2. First indication of the *open spireme*.
- Fig. 3. A further opening of the knot.
- Fig. 4. One ring element coming into view showing the separating achromatic bridge of the primary constriction.
- Fig. 5. A further development with tetrads constricting.
- Fig. 6. A further development of figure 5. Constriction bridges evident.
- Fig. 7. Three ring elements defined. Independent ends with achromatic bridges.
- Fig. 8. Four ring elements clearly visible.
- Fig. 9. Achromatic bridge of the primary constriction in the act of parting to develop tetrads. Enlarged as plate 1.
- Fig. 10. An octad closed ring.
- Fig. 11. An octad linkage separating on the first metaphase showing fiber insertions.
- Fig. 12. A closed octad ring on the metaphase. Note the comparative size of the tetrads to the left.
- Fig. 13. A continuous spireme linkage of *Rhoeo* on the first metaphase spindle.

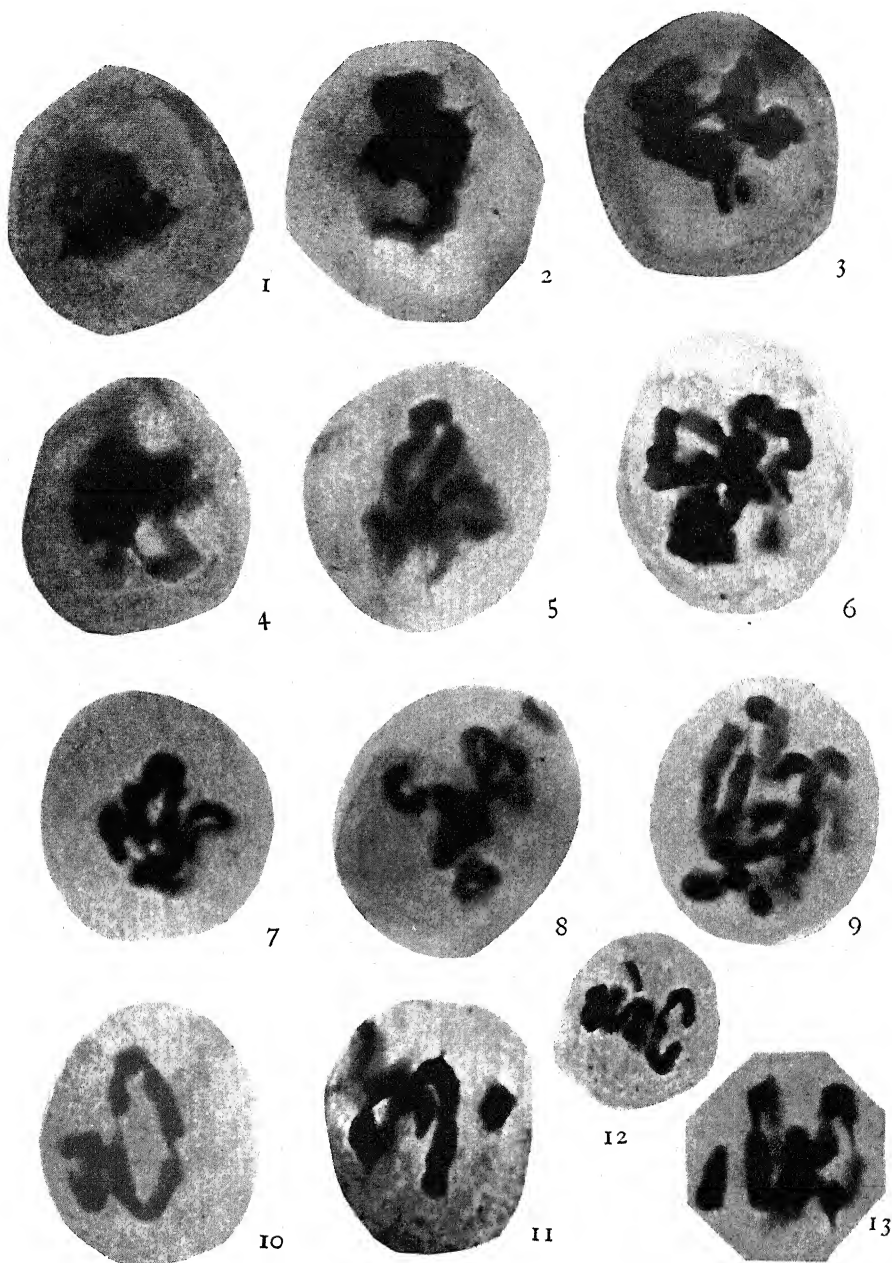
PLATE 3

Figures 14, 15, 17, and 18 from aceto-carmin. Others fixed in Flemming's medium fluid. Material, *Tradescantia*.

- Fig. 14. Metaphase and anaphase initials of the first reduction spindle. Note the extreme left element enlarged as text figure 5.
- Fig. 15. A closed tetrad ring (southwest element) in the course of separating the outside ends before the secondary constriction is active.
- Fig. 16. A closed octad ring in the course of being oriented for separation on the first metaphase spindle.
- Fig. 17. An open octad linkage, upper center.
- Fig. 18. Anaphase dyads resulting from the persistence of the tertiary constriction and side-by-side approximation of the chromatids.
- Fig. 19. Cone-shaped insertion of the fibers. It indicates the greater tenacity of the secondary constriction in contrast to those unions caused by flexing and the association of the formerly free ends.
- Fig. 20. An opening tetrad indicating the stoutness of the spindle fibers.
- Fig. 21. Some of the openings more advanced.



SANDS: PERIGENESIS



SANDS: PERIGENESIS



14



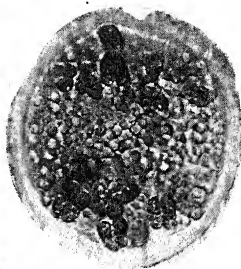
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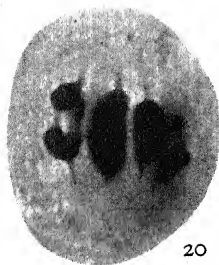
16



17



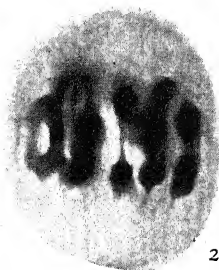
18



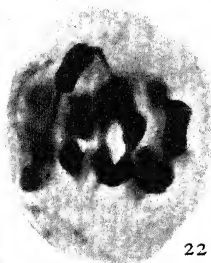
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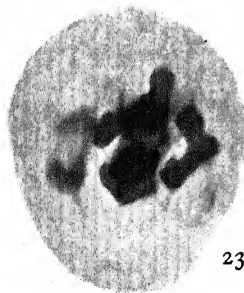
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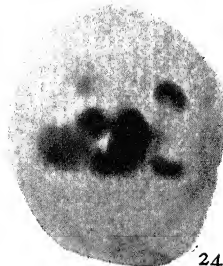
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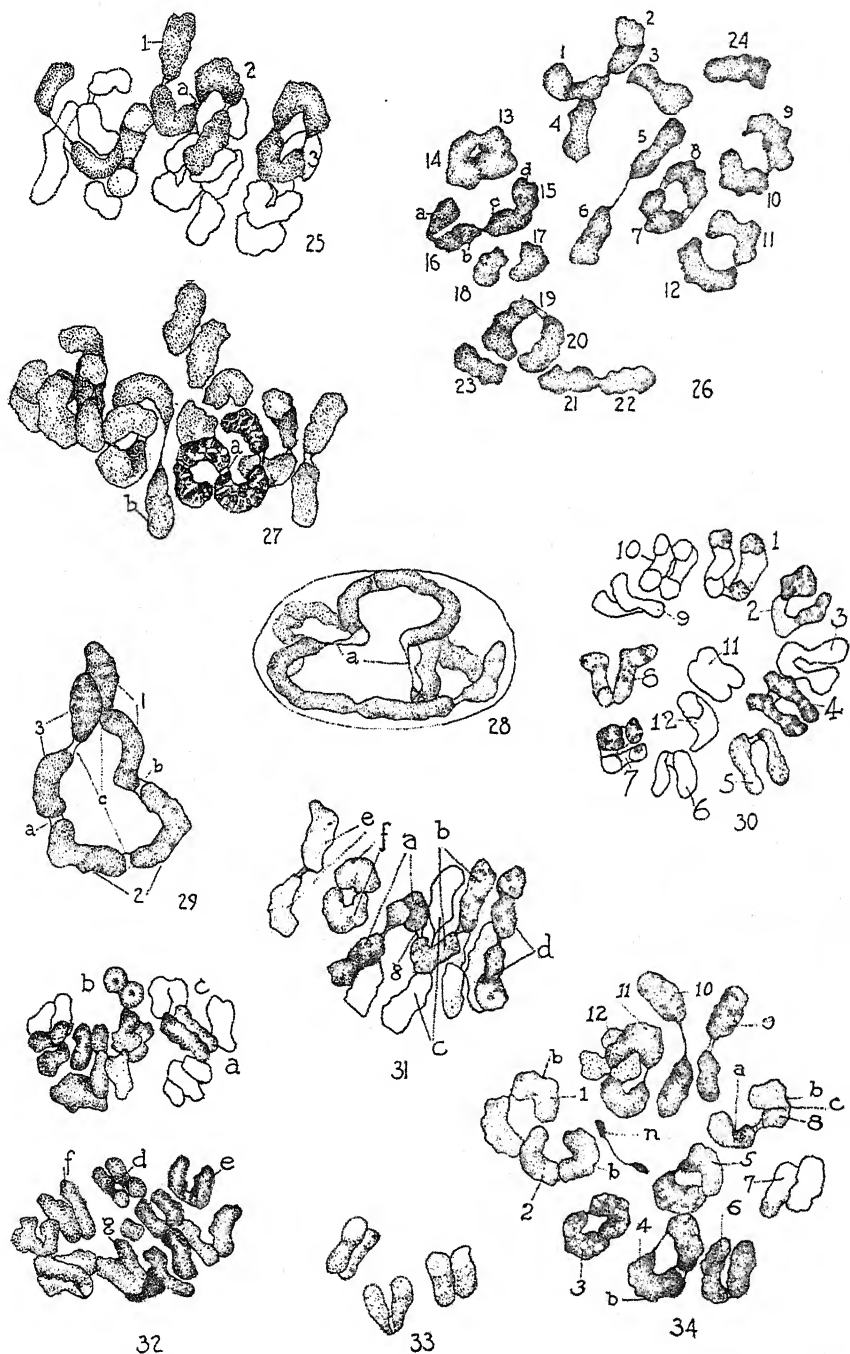


23



24

SANDS: PERIGENESIS



SANDS: PERIGENESIS

Fig. 22. Linkages carried to the metaphase plate of the first spindle.

Fig. 23. An advanced stage of figure 21.

Fig. 24. A more extreme separation of the dyads with the tertiary constriction not yet well marked. The achromatic bridge of the secondary constriction persistent to form the so-called traction fiber.

PLATE 4

All figures are from *Tradescantia* fixed by aceto-carmines except figure 29, which is of *Rhoco*.

Fig. 25. An octad linkage overlying the other elements. The primary constriction persistent at (a).

Fig. 26. A metaphase of the first reduction spindle and a drawing of the photograph figure 14, plate 3. The tetrads are of various stages of the separation with the extreme represented by elements 23 and 24. Note the lack of synchrony for example between elements 13 and 14 and 5 and 6.

Fig. 27. The octad of figure 25 with the chromosomes more clearly marked.

Fig. 28. A hexad abstricting at (a).

Fig. 29. A *Rhoco* linkage of three tetrads on the first metaphase. Primary constrictions at (a) and (b). Secondary constrictions not clearly evident.

Fig. 30. Polar crown showing wide separation of the chromatids still bound by the persistent tertiary constriction. Element 4 shows the bridge insertion to have slipped to a sub-terminal position. An extreme expression of this leads to sharp X forms.

Fig. 31. First indications of the tertiary constriction in the octad linkage (a + b). More clearly seen in the outside unit of a.

Fig. 32. Side view of anaphase dyads. Note the end view at (b) indicating the achromatic medulla.

Fig. 33. A drawing of the three lower elements of figure 18, plate 3. Compare with figures 30 and 32 and element 16, figure 26.

Fig. 34. The elements of 12 rings (tetrads) identified at *n*, an extruded fragment (cf. McClintock, 1931, *b*, fig. 26). Elements 6 and 7 comparable to Farmer and Moore's (1905) bivalents. Tertiary constrictions beginning at *b*, elements 1, 2, 4, and 8 (cf. also Blakeslee, 1929, fig. 19).

THE EVOLUTIONARY STATUS OF PLANT FAMILIES IN RELATION TO SOME CHEMICAL PROPERTIES¹

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(Received for publication April 6, 1933)

The object of this paper is to show that when the plant families which contain fats, volatile oils, and alkaloids are first separated according to climate of habitat some chemical and physical properties of these substances vary in accordance with the degree of evolution of the plant families containing them, and that the probability is that the more highly organized the plant the more complex are its chemical products.

DATA

Some 318 fats, 232 waxes, 938 volatile oils, and 299 alkaloids have been analyzed from 83, 84, 87, and 57 plant families, respectively. As there are 295 plant families, some 30 per cent of them have been analyzed for these substances. The families that produce these materials may be divided into climatic groups as follows: tropical, tropical-subtropical, subtropical, subtropical-temperate, temperate, and widely distributed. By far the most of these families are to be found in the tropical, temperate, or widely distributed groups. The tropical and temperate families that contain alkaloids, fatty oils (glycerides), and volatile oils are to be found in tables 1, 2, 3, and 4. These two zones (tropical and temperate) are chosen because they represent extremes in climatic difference.

The list of tropical alkaloid families (table 1) begins with the Palmae and Stemonaceae, closes with the Acanthaceae and Rubiaceae, and includes many quite evenly dispersed families between these limits. For example, there are two families with botanical serial numbers below 1000, two between 1000 and 2000, five between 2000 and 3000, two between 3000 and 4000, two between 5000 and 6000, three between 6000 and 7000, one between 7000 and 8000, and one between 8000 and 9000. Consequently the group presents a representative cross section of all of the tropical families.

Similar wide and representative dispersals of data are shown in the tables of glycerides and volatile oils (tables 2, 3, and 4). These dispersals are graphically portrayed in the scatter diagrams (fig. 3, 4, 5) and show that any additional data will fall within the scope of the statistics already obtained.

¹ Presented by the author before the Botany Club, University of Chicago, March 7, 1932.

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From these tabulated data it has been definitely determined that there is a consistent variation in some of the chemical and physical properties of these materials according to the tropical and temperate climates in which they are produced. For instance, it has been found that the tropical and subtropical glycerides have higher melting points and lower iodine values (i.e., they are more saturated) than the fatty oils of temperate climates (McNair, 1929). This finding is supported by the evidence of Hilditch (1928), who in analyzing fat constituents found specific acids for four plant families—namely, lauric (mol. wt. 200, m. p. 48°C.) for the Palmae, myristic (mol. wt. 228, m. p. 58°C.) for the Myristicaceae, erucic (mol. wt. 338, m. p. 33.5°C.) for the Cruciferae, and petrosilinic (mol. wt. 282, m. p. 14°C.) for the Umbelliferae. As the Palmae and Myristicaceae are tropical and the Cruciferae and Umbelliferae are temperate, the average molecular weight of the tropical families, 214, is lower than that of the temperate, 310. At the same time the average melting point of these tropical acids, 53°, is higher than that of temperate, 23°.

In the volatile oils it has been noted, according to the refractive indices and specific gravities, that tropical volatile oils have compounds of greater saturation. This coincides with the greater saturation of tropical glycerides. The molecular weights and melting points of the constituent alcohols, acids, and esters of these volatile oils are lower for the tropically formed substances (McNair, 1932).

The alkaloids are interesting illustrations of the general tendency toward change in substances formed in different climates. The average molecular weights of these nitrogenous bodies are lower in the tropics, while their average melting points are lower in the temperate zone. These observations have been found true whether comparisons were made between the 67 alkaloids of proven molecular structure (McNair, 1931) or when the total of 299 alkaloids was used whose chemical properties have only been partially established. It is not known how many more families contain alkaloids, but the examples of the 67 and the 299 alkaloids form unique evidence for the confirmation of the uniform variation in chemical properties with climate. These climatic changes in physical and chemical properties of glycerides, volatile oils, and alkaloids indicate in general that more complex compounds are formed in temperate than in tropical plant families. The fact that these changes are generally consistent causes one to deduce that a sufficient number of chemical analyses of the determinant substances have been made from sufficiently representative ranges of evenly dispersed families and that any additional analyses will fall in the groups already established and will only more strongly confirm the conclusion already determined.

DEFINITIONS AND SPECIFICITY OF CHEMICAL COMPOUNDS

In any one climate any physical or chemical changes in specific chemical products may perhaps serve as an index of the degree in evolution of the plant family in which these changes take place.

It is necessary that the chemical substances used for this purpose be as specific as the plant unit used, be it family, genus, or species. For this reason there have been chosen for this paper alkaloids, glycerides, and volatile oils.

Alkaloids. Vegetable alkaloids are basic nitrogenous substances often possessing some important action in animal physiology. Most alkaloids are made up of carbon, hydrogen, oxygen, and nitrogen, have large molecular weights, are crystalline and non-volatile. A few, however, such as coniine and nicotine, do not have oxygen in their composition but consist entirely of carbon, nitrogen, and hydrogen. These likewise are volatile liquids. The majority of alkaloids are sparingly soluble in water, although they dissolve in alcohol, chloroform, ether, and other organic solvents. The liquid alkaloids, as coniine and nicotine, are readily soluble. Alkaloids generally form salts with acids which dissolve in water and crystallize well. A large number of alkaloids have a very bitter taste and are excessively poisonous. Many find use in medicine, and their value in this respect can hardly be overrated.

The chemical structure of numerous alkaloids is that of tertiary aromatic bases, but the constitutions of many of them have not yet been completely determined. It is certain, however, that some are derived from pyridine, quinoline, or isoquinoline.

Out of a total of some 299 known alkaloids only 19, or 6.3 per cent, are found in more than one plant family. It is interesting to note that the average molecular weights of the alkaloids which are found in more than one plant family are lower than the average molecular weights of the other alkaloids which are confined to individual families in the same climate of habitat. That is, these substances of more general distribution are simpler in chemical composition than those substances produced by individual families.

When different alkaloids occur in the same plant family, each individual alkaloid is generally confined to a single genus. If more than one alkaloid is located in a genus, their particular nitrogenous bases are usually closely related. For instance, the various aconitines have been separated only from members of the genus *Aconitum*. *Aconitum* is noteworthy in giving a new chemical species of aconitine for each new botanical species analyzed, although all the aconitines are apparently closely related.

Alkaloids in the same species generally have the closest chemical relationship to one another. Frequently they form a homologous series, often they are isomers, and sometimes stereoisomers. In other instances the difference between these compounds is only in the quantity of hydrogen or oxygen which they possess; accordingly reduction or oxidation will convert one into another. This condition has led Biddle (1913) to state that "there seems to be thus an intimate connection between the properties on which the classification of plants is based and those which naturally determine the classification of alkaloids."

Glycerides. Glycerides are esters formed from glycerol and fatty acids. They are by far the chief constituents of the fats or fatty oils. The term

glyceride is used here instead of fat or fatty oil because it is more definite and avoids the confusion between fatty oil and volatile oil.

Glycerides often provide specific physical and chemical properties of taxonomic value, through comparisons of iodine numbers, saponification values, and specific gravities.

There is close chemical and physical agreement between the glycerides of the different species of a genus. The glycerides of most of the smaller families are in close intrafamilial agreement, while those of the larger families are often in better agreement if they are considered in tribal groups (McNair, 1929, 1930).

Seed fats from plants belonging to the same or nearly allied botanical families often contain similar, and to a certain extent specific, mixtures of fatty acids. This has been definitely shown to exist in the Palmae, Myristicaceae, Cruciferae, and Umbelliferae (Hilditch, 1928). Other acids, of course, are also present, such as oleic and linoleic acids usually in fair to considerable proportions, and also minor amounts of such acids as palmitic, arachidic, or lignoceric; but the four acids, lauric, myristic, erucic, and petroselinic, stand out quite definitely in their value and proportions in the respective cases of the four families, Palmae, Myristicaceae, Cruciferae, and Umbelliferae.

Volatile oils. Volatile oils, also called essential, ethereal, or aromatic oils, are aromatic volatile substances of an oily nature usually obtained by the distillation of vegetable products with steam. They are generally liquid, though sometimes semi-solid at ordinary temperatures, slightly soluble in water, soluble in alcohol, ether, benzene, light petroleum, and most organic solvents. They are found in all parts of the plant or tree, some occurring in the woody stems or roots, others in the bark, leaves, flowers, and fruits. Many essential oils are complex mixtures, containing constituents belonging to various classes of organic compounds, as hydrocarbons, alcohols including phenols, aldehydes, acids, esters, phenol esters, ketones, lactones, quinones, oxides, bases, sulphides, mercaptans, nitriles, and isothiocyanates. In some cases the oil consists almost entirely of one constituent—e.g., bitter almond oil (benzaldehyde), black mustard oil (allylisothiocyanate), and wintergreen oil (methyl salicylate). They are distinguished from fatty oils by their acrid taste, volatility, aromatic odor, solubility in alcohol, non-greasiness, and non-glycerol content.

Just as the odor of the flower of the lemon is distinct from the odor of that of the orange, the rose from the apple, and the violet from the grape, so the complex mixtures of the various chemical compounds present in most volatile oils tend greatly to become individual in character for each plant species and to have also more general specific properties for most plant families.

It is thus evident from the descriptions presented above that the three classes of chemical compounds—alkaloids, glycerides, and volatile oils—are

sufficiently specific to be used complementary to morphological characters in the classification of plants.

CHEMICAL COMPOUNDS AS SPECIES CONSTITUENTS

The volatile oils and alkaloids have already been used as aids in the determination of species.

Baker and Smith (1920) in their 30 years' study of the genus *Eucalyptus* concluded with Bentham that "the groups pass very gradually into each other through intermediate forms," but the individual species shows a comparative constancy of specific characters throughout its known geographical distribution. Not only is this the case with the botanical characters, but also in their chemical constituents. Baker and Smith divided the genus into eight different chemical groups in accordance with the presence, absence, or percentage composition of several constituents of their volatile oils. These components are: alcohols (cineol or eucalyptol); hydrocarbons (the terpenes, pinene and phellandrene); ketones (piperitone); and aldehydes (aromadendral). Such chemical changes are accompanied by characteristic venations of the leaves. Corresponding to these well-marked differences, other changes have also taken place, which have become discernible in the varying barks and woods of the Eucalypts, as for instance, representing the several groups, there are the "Stringybarks," the "Ironbarks," the "Smoothbarks," or "Gums," the "Boxes," the "Ashes," etc. The exudations or kinos have also varying chemical characters, which are as constant as those of the oils.

Baker and Smith (1920) present an evolutionary sequence for the Eucalypts. In this diagram the most primitive produce volatile oils with pinene, but contain practically no phellandrene or cineol. Higher groups contain phellandrene, cineol, and other compounds with or without pinene. In this classification the hydrocarbons are considered as indicative of more primitive plants than their oxidation products—alcohols, aldehydes, and ketones.

Volatile oils are again used by Baker and Smith (1910) for the classification of the genus *Callitris* in their study of Australian pines. They found the genus to divide itself into eighteen species which fall into three groups. These three groups are based on hydrocarbon content as follows: In group 1 the predominant limonene in the leaf oils is dextro-rotatory; in group 2 the predominant limonene in the leaf oils is laevo-rotatory; and in group 3 the principal terpene in the leaf oils is pinene. These three groups have morphological and anatomical counterparts: Group 1 has more or less tuberculate fruits, and a convex dorsal leaf surface, while sclerenchymatous or stone cells are mostly absent in the leaf tissue; group 2 has generally smooth fruits and angular or rounded dorsal leaf surfaces, and the sclerenchymatous cells in the leaf tissue are more numerous than in the species in group 1; group 3 has generally smooth fruits and angled dorsal leaf surface, and sclerenchymatous cells occur plentifully in the leaf tissue.

The concomitant changes in the morphology and chemistry of *Callitris*

species may have been caused by selective influences. Territorial selection by the species themselves and the chemical peculiarities of certain situations and sorts have undoubtedly had marked influences upon the location chosen by the young trees, where it would be possible for them to establish themselves and flourish. In New South Wales there are districts where the species of *Callitris* do not naturally occur, and this is apparently due to the peculiarities of these localities which make them unsuited for their natural establishment. Portions of this state known as the "Black Soil Plains" may particularly be mentioned in this connection, and although some of the species approach these districts on all sides, yet they do not invade them. To the species of *Callitris* they evidently are forbidden fields.

The results obtained from the study of the Eucalypts, growing *under natural conditions* in Australia, showed a remarkable constancy in the oil constituents of the several species. It was found during this investigation by Baker and Smith (1920) that any well-defined species of *Eucalyptus* would always give practically the same products, not only in oil constituents, but also in other chemical peculiarities. Subsequent investigation by Baker and Smith has added considerably to our knowledge in this direction, and no marked differences in the general character or constituents of the oil distilled from any one species have yet been found, no matter in what part of the country the trees were grown. It might, of course, be feasible to bring about alteration in the chemical constituents of the plant by artificial methods, extending over a sufficiently long period, but under natural conditions such alterations as have taken place must have been slow, although eventually succeeding in establishing such marked differences, both in botanical and chemical characters, as have warranted their separation for classification purposes into distinct species.

It was felt that the importance of this question required extended investigation with other large Australian genera besides the Eucalypts, and for this purpose material of some of the species of *Callitris* has been obtained from various localities very far apart, and during several years. It will be seen from the results recorded under the several species, particularly *C. glauca*, that the chemical constituents of the essential oils of the species of *Callitris* are remarkably constant when grown under *natural conditions*, notably their ester content. The tannins in the barks are also in agreement, so that it is possible by chemical reactions to distinguish the tannin of *C. glauca* and allied species from that of *C. calcarata*. In fact, all the specimens Baker and Smith (1910) examined answered to these distinguishing tests. Spreading over such a large extent of territory as do the species of *Callitris*, and being all the time subjected to the diverse climatic and other direct influences found in such a large continent, it is perhaps surprising that there are so few well-defined species of *Callitris* in Australia.

The constancy of chemical characters found to occur in the several species has thus been of considerable help in deciding the differentiation governing

their classification. It has been possible to correlate the differences of alteration in the species themselves, and so allot specific values to those botanical characters which evidently have been established under exactly similar conditions and influences as those which fixed their chemical differences. The determination of the possible changes which may be brought about by specific treatment of the several species is left to other investigators. In this work only those plants established under natural conditions have been dealt with, and the results which have thus far been obtained with these do not warrant the supposition that alterations are now taking place with sufficient rapidity to enable one to discern them. Evidently time is one of the main factors in these alterations, and human life is too short for their discernment. Results having been obtained from nearly the whole of the genus *Callitris*, gathered throughout the whole range of its distribution, it has been possible to formulate conclusions which could not have been advanced if the study had been restricted to any one species.

In both *Callitris* and *Eucalyptus* the leaves are persistent during the whole year, and the flowering period seems to play a comparatively small part in the chemical changes of the essential oils in both genera, so that the results which have been obtained in Europe by the study of those chemical changes which take place in the oils of such plants as *Mentha piperita*, *Pelargonium odoratissimum*, etc., during their several periods of growth and flowering appear scarcely to assist when applied to such genera as *Callitris* and *Eucalyptus*. The changes which occur in the oils of these plants seem to be specific, and no periodic alterations of a very marked character have been found in any one well-defined species, so that only slight differences in the constitution of the essential oils are perceptible during any part of the year. Supposed differences in this direction have often been found to be due more to differences of opinion as regards nomenclature than to the alterations in the constituents of the specific species themselves. It is thus seen that the chemical products manufactured by individual species both in *Callitris* and *Eucalyptus* have a considerable systematic value, and their study, therefore, becomes of some importance when seeking for specific differences in plant classification.

The conditions largely of a chemical nature which succeeded in establishing such definite alterations also brought about marked differentiations in the character of the species themselves. This conclusion may be supported by such well-defined species as *Callitris glauca* and *C. calcarata*, the former growing almost exclusively on the plains, the latter species on the hills. In districts where both occur, it is possible to follow roughly the margin of the location of either species on the map, and at the same time indicate fairly well the contour of the hilly country. Wherever *C. glauca* occurs, its chemical peculiarities are found to be specific in all directions, and markedly so in contradistinction to those of *C. calcarata*, or vice versa. It seems necessary, therefore, that the conditions which were originally responsible for the establishment of these characteristic chemical peculiarities should persist if

the results are to be of permanent value. It is thus reasonable to consider that the well-defined chemical constituents of a plant are, for all practical purposes, as systematically valuable as the morphological characters, and that, when all this evidence is correlated, the species so founded will be established with a considerable degree of stability. *C. Tasmanica*, growing in Tasmania, gave an oil which agreed entirely with that from the same species growing on the highlands of New South Wales, hundreds of miles away. Evidently here the natural conditions under which the species had become established were uniform. The morphologically closely agreeing species *C. rhomboidea* of the coast of New South Wales was found to differ in its chemical characters from those of *C. Tasmanica*.

Besides the volatile oils, the alkaloids have been shown to display chemical and physical properties which are closely allied to the morphological characters of the plants in which they are found. The genus *Aconitum* furnishes an excellent example, showing a new chemical species of aconitine for each new botanical species analyzed, although all of the aconitines are closely related. Carr (1912) has summarized these nitrogenous compounds as follows: *Aconitum Napellus* (monk's hood) of Europe has as its principal alkaloid aconitine with a formula of $C_{34}H_{47}O_{11}N$ and a melting point of $197^{\circ}C$.; *A. Fischeri* of Japan produces japaconitine, $C_{34}H_{49}O_{11}N$, m. p. 204° ; *A. chamanthum* Stapf of India gives indaconitine, $C_{34}H_{47}O_{10}N$, m. p. $202-203^{\circ}$; *A. deinorrhizin* Stapf (*A. ferox* of some authors) of India yields pseudoaconitine, $C_{36}H_{50}O_{12}N$, m. p. $211-212^{\circ}$; *A. spicatum* Stapf (*A. ferox* of some authors) of India furnishes bikhaconitine, $C_{36}H_{51}O_{11}N$, m. p. $113-116^{\circ}$; and *A. japonicum* Thunb. (*A. Fischeri* of some authors) of Japan forms jesaconitine, $C_{40}H_{51}O_{12}N$.

THE NATURAL OR EVOLUTIONARY SYSTEMS OF PLANTS

Several systems of plant classification have been developed, such as those of Bentham and Hooker (1862), Engler and Prantl (1897), Bessey (1915), Rendle (1925), Hutchinson (1926), and Mez (1925). These systems vary in the arrangement of their plant families, not only as to the relative positions of the families within the systems, but also as to the families chosen for origin and termini. They all agree, however, in having more primitive families as origins and more highly organized families as termini. For convenience Engler and Prantl's system is chosen to illustrate the relative general evolutionary position of the plant families in respect to the chemical compounds formed by these families. In this paper the serial numbers given these families by DeDalla Torre and Harms (1907) have been used.

In giving serial numbers to the various plant families in the Engler and Prantl system, DeDalla Torre and Harms begin with the most primitive plants and continue to the most highly organized plants. The arrangement of plant families in accordance with their evolutionary positions assumes a tree-like form with the more primitive families at the base of the tree and the

most highly organized families at the tips of the branches, the topmost families on the tree representing the most highly evolved plants. It might appear at first glance that a linear system of numbering would not truly represent the proper relative positions of the various families in their order of evolution. Such, however, is not the case, as the following examples will show. For instance, the gymnosperms are treated first and have smaller numbers than the angiosperms. In the angiosperms the monocotyledons have lower numbers than the dicotyledons. In the dicotyledons the members of the subdivision of Archichlamydeae have lower numbers than have the Metachlamydeae. Of the Metachlamydeae the order Diapensiales is succeeded by the Ebenales, Contortae, Tubiflorae, Rubiales, and Cucurbitales. Each order has the most primitive family as its lowest number and the latest family has the highest number. In the DeDalla Torre and Harms numbering of the various Engler and Prantl genera it is as though the evolutionary tree were stripped of its branches and each branch, beginning with the lowest on the tree, were laid tip to base in a horizontal line which terminates with the highest branch.

In order for evolution to have taken place in a tree-like form, it is necessary that a lower branch be older (more primitive) than the one above it. Consequently each branch base represents a stage in progressive development which can be correctly expressed by serial numbers. Likewise the apical tips of the branches typify steps in the evolutionary progress of the plant families which also can generally be accurately shown with respect to each other by serial numbers. It may be, however, that the tip of a lower branch portrays a higher stage than the base of the branch next above it. If such is the case, it is nevertheless true that this higher stage is not higher than the base of the branch to which the tip belongs. Therefore the average serial number of a lower branch must generally be lower than the average serial number of the branch just above it. Consequently, although the serial numbers of DeDalla Torre and Harms constitute a linear system, they are nevertheless sufficiently representative of the tree-like natural system of plant evolution for our purpose. The position of the orders on figures 1, 3, 4, and 5 shows this to be true. Should an individual family be wrongly placed in the system, the use of the average botanical number of groups of families tends to eliminate such error.

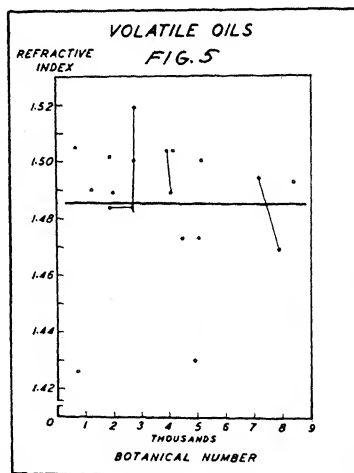
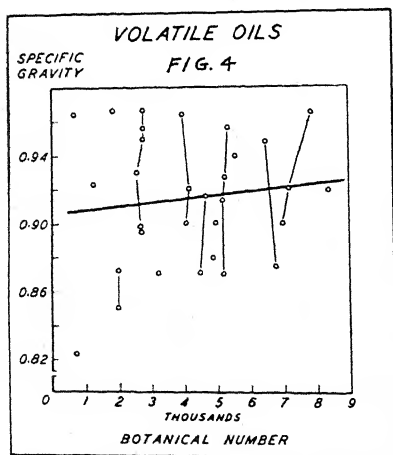
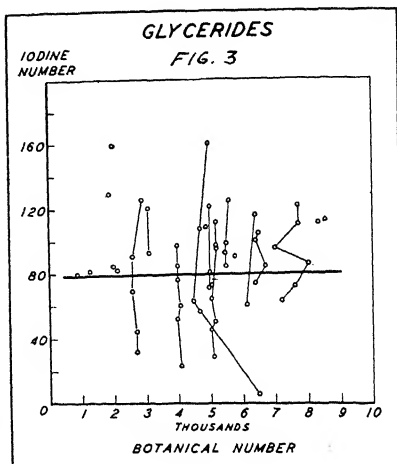
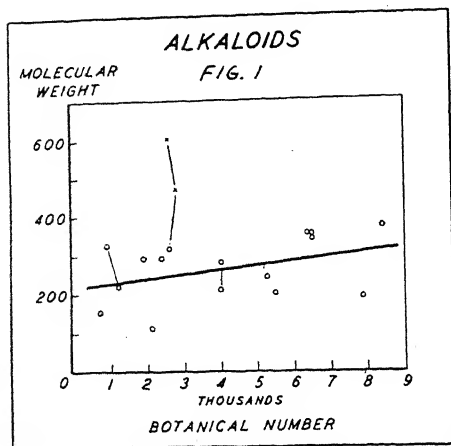
EVOLUTIONARY STATUS OF FAMILIES IN RELATION TO SOME CHEMICAL PROPERTIES

Alkaloids

If the alkaloids be first separated according to the habitat climates of the plant families producing them, it becomes apparent that the alkaloids of the highest average molecular weight are produced by temperate plants and that those with the lowest are obtained from tropical families (McNair, 1931). An inspection of table 1 makes this obvious.

In figure 1 the data from the tropical families of table 1 have been ar-

ranged in a scatter diagram. Each point represents the average molecular weight of the alkaloids contained in a certain family, which family is indicated by its numerical position in the Engler and Prantl system. A vertical line connecting various points shows the families of an order.



To determine in this group whether the alkaloids of highest molecular weight are produced by the families highest in evolution, a straight line is used. A straight line is chosen because a straight line constitutes the best means of showing a trend. There is one and only one straight line which fits most accurately the plotted data. The constants of this line of best fit may be determined by the method of least squares (Mills, 1924).

It is assumed that the data of the Lauraceae and Anonaceae (Nos. 17 and 18) are in error or form a separate unit, as their points on the diagram

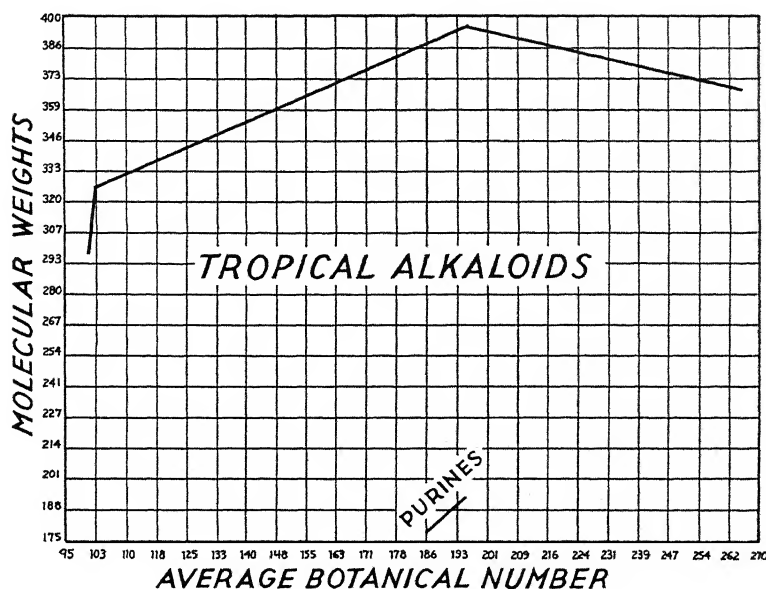


Fig. 2

are far removed from the others. The data from all the rest are used in the calculations. It is considered that the botanical numbers are free from any error, and therefore they are used as independent variables. The equation obtained is

$$y = 215.96 + 0.011544x$$

where x indicates the botanical numbers and y the average molecular weights of the alkaloids (table 1). This equation has a positive slope ($+0.011544$) which shows the line to have a decidedly upward trend.

If it is assumed that the molecular weights of the alkaloids are free from any error, and are used as independent variables, the equation is

$$x = 901.12 + 12.2971y$$

which also has a positive slope.

If both the botanical numbers and the molecular weights are considered as not free from error, the trend would have a similar nature.

It can therefore be definitely stated that the higher the tropical plant family is in evolutionary development, the greater will be its tendency to form alkaloids of large average molecular weight.

This can also be demonstrated with the alkaloids that are formed by more than one family (fig. 2). The climatic disposition of these alkaloids is determined by majority rule and their position on the evolutionary scale obtained by averaging the botanical serial numbers of the families producing them, as the following examples will show.

Caffeine. Caffeine, molecular weight 194, is found in 3 tropical families,

TABLE I. *Alkaloids—their molecular weights and botanical and climatic distribution*

Average molecular weight y	Botanical no. (DeDalla Torre and Harms) x	Family (Engler and Prantl)	Order (Engler and Prantl)
Tropical			
121	2070	Loranthaceae	Santalales
144	667	Palmae	Principes
188	7906	Acanthaceae	Tubiflorae
197	5558	Myrtaceae	Myrtiflorae
208	3980	Zygophyllaceae	Geraniales
221	1250	Dioscoreaceae	Liliiflorae
239	5377	Caricaceae	Parietales
269	3956	Erythroxylaceae	Geraniales
285	1859	Piperaceae	Piperales
289	2387	Aizoaceae	Centrospermae
314	2567	Menispermaceae	Ranales
325	939	Stemonaceae	Liliiflorae
344	6460	Loganiaceae	Contortae
351	6549	Apocynaceae	Contortae
352	6418	Symplocaceae	Ebenales
374	8381	Rubiaceae	Rubiales
468	2782	Lauraceae	Ranales
597	2565	Anonaceae	Ranales
Temperate			
143	6063	Umbelliferae	Umbelliflorae
215	5401	Cactaceae	Opuntiales
330	2558	Berberidaceae	Ranales
355	2852	Papaveraceae	Rhoeadales
446	2961	Cruciferae	Rhoeadales
543	2539	Ranunculaceae	Ranales

Rubiaceae serial number 8381, Sapindaceae 4734, and Sterculiaceae 5090; one tropical-subtropical family, Theaceae 5144; and one widely distributed family, Aquifoliaceae 4614. Caffeine is therefore considered as tropical. Its botanical serial number, the average of those appended, is $27963 \div 5 = 5592$.

Brucine. Brucine, molecular weight 394, is found in two tropical families: the Loganiaceae 6460 and the Simarubiaceae 4106. It therefore has a botanical serial number of $10566 \div 2 = 5283$.

Bebeerine. Bebeerine, molecular weight 297, has been discovered in three tropical families, the Menispermaceae 2567, Lauraceae 2782, and Hernandiaceae 2829. Its botanical serial number is consequently $8178 \div 3 = 2726$.

Laurotetanine. Laurotetanine, with a molecular weight of 327, has been isolated from two tropical families, the Lauraceae 2782, and the Hernandiaceae 2829. The average botanical number for this alkaloid is the average of 2782 and 2829, or 2805.

Theobromine. Theobromine, molecular weight 180, is a purine body closely related to caffeine. It is found in one tropical family, the Sterculiaceae 5090, and one tropical-subtropical family, the Theaceae 5144. Its average botanical number is $10234 \div 2 = 5017$.

Yohimbine. Yohimbine, molecular weight 368, has been separated from

two botanical families, the Rubiaceae 8381, and the Apocynaceae 6549. The botanical number for this substance is $14930 \div 2 = 7465$.

According to this method alkaloids which occur in two or more plant families, when first climatically divided, may be subdivided according to their average botanical serial numbers. When this is done, it is evident that the tropical alkaloids of higher molecular weight are formed by plant families of higher average on the evolutionary scale.

Glycerides

The glycerides or fatty oils which are found in the seeds and other parts of many plants may also be divided according to climate of origin (McNair, 1929, 1930). The individual glycerides may be arranged in relation to their iodine numbers as in table 2 (Lewkowitsch, 1922; Grün and Halden, 1929). The iodine value of oils and fats is obtained by allowing a measured amount of an iodine solution to act on the oil or fat. It has been found that a considerable amount of iodine is absorbed by the fatty acids present. The percentage of iodine absorbed is taken as the iodine value.

In table 2 the temperate fatty oils (glycerides) have been separated from those produced by tropical plant families. It is apparent from this table that temperate fatty oils have higher average iodine values than tropical.

In figure 3 the data of the tropical glycerides from table 2 have been arranged in a scatter diagram. Each point represents the average iodine number of the glycerides obtained from a certain family, which family is indicated by its numerical position in the Engler and Prantl system. A vertical line connecting various points shows the families of an order.

To determine the trend of these points, a straight line is used as in the previous case of alkaloids (fig. 1). The constants of this line of best fit may be determined by the method of least squares (Mills, 1924). It is assumed that the botanical numbers are free from any error, and therefore they are used as independent variables. The resulting equation is

$$y = 78.98834 + 0.00013287x$$

where x indicates the botanical numbers and y the average iodine numbers of the glycerides. This equation has a positive slope ($+0.00013287$) which shows the line to have an upward trend.

If both the botanical numbers and the molecular weights are considered as not free from error, the trend would have a similar nature.

It can therefore be definitely stated that the higher the tropical plant family is in evolutionary development the greater will be its tendency to produce glycerides of large average iodine numbers (and also the lower will be their melting points).

TABLE 2. *Glycerides—their iodine values and botanical and climatic distribution*

Iodine no. y	Botanical no. (DeDalla Torre and Harms) x	Family (Engler and Prantl)	Order (Engler and Prantl)
Tropical			
6.69	6446	Salvadoraceae	Sapindales
23.1	4265	Vochysiaceae	Geraniales
28.45	5211	Dipterocarpaceae	Parietales
28.9	667	Palmae	Principes
31.03	2737	Myristicaceae	Ranales
44.93	2782	Lauraceae	Ranales
45.68	5135	Caryocaraceae	Parietales
50.7	5377	Caricaceae	Parietales
52.47	4106	Simarubiaceae	Geraniales
57.97	4734	Sapindaceae	Sapindales
59.28	6370	Sapotaceae	Ebenales
60.40	4140	Burseraceae	Geraniales
63.28	4543	Anacardiaceae	Sapindales
64.15	7178	Verbenaceae	Tubiflorae
65.57	5115	Ochnaceae	Parietales
68.7	2567	Menispermaceae	Ranales
71.23	5023	Bombacaceae	Malvales
71.6	5162	Guttiferae	Parietales
72.48	7662	Bignoniaceae	Tubiflorae
74.35	6460	Loganiaceae	Contortae
76.14	4186	Meliaceae	Geraniales
79.23	5090	Sterculiaceae	Malvales
80.9	684	Araceae	Spathiflorae
81.07	1324	Zingiberaceae	Scitamineae
82.93	2148	Olacaceae	Santalales
84.27	6792	Asclepiadaceae	Contortae
84.75	2016	Proteaceae	Proteales
84.8	5537	Combretaceae	Myrtiflorae
85.8	3953	Humiriaceae	Geraniales
86.35	7906	Acanthaceae	Tubiflorae
89.4	2665	Anonaceae	Ranales
91.8	5855	Araliaceae	Umbelliflorae
92.4	3128	Moringaceae	Rhoeadales
92.5	5535	Rhizophoraceae	Myrtiflorae
95.05	5275	Flacourtiaceae	Parietales
96.	5260	Cochlospermaceae	Parietales
96.25	6968	Convolvulaceae	Tubiflorae
97.02	3980	Zygophyllaceae	Geraniales
98.23	5502	Lecythidaceae	Myrtiflorae
98.74	6420	Oleaceae	Contortae
104.6	2364	Phytolaccaceae	Centrospermae
105.61	6549	Apocynaceae	Contortae
108.3	4665	Staphyleaceae	Sapindales
109.61	4916	Vitaceae	Rhamnales
109.8	7768	Pedaliaceae	Tubiflorae
110.77	8381	Rubiaceae	Rubiales
111.55	5249	Bixaceae	Parietales
112.5	8590	Cucurbitaceae	Curcubitales
116.8	6406	Ebenaceae	Ebenales
119.5	3089	Capparidaceae	Rhoeadales
121.5	4950	Tiliaceae	Malvales
122.5	7784	Martyniaceae	Tubiflorae
124.78	5558	Myrtaceae	Myrtiflorae
126.1	2829	Hernandiaceae	Ranales
129.	1859	Piperaceae	Piperales
158.5	1973	Moraceae	Urticales
160.	4856	Balsaminaceae	Sapindales

TABLE 2.—*Continued.*

Temperate			
78.4	2551	Lardizabalaceae	Ranales
85.3	1887	Betulaceae	Fagales
96.5	4720	Aceraceae	Sapindales
103.8	6063	Umbelliferae	Umbelliflorae
110.7	2961	Cruciferae	Rhocadales
116.3	5401	Cactaceae	Opuntiales
127.8	1882	Juglandaceae	Juglandales
137.1	8515	Caprifoliaceae	Rubiales
138.5	8116	Plantaginaceae	Plantaginales
139.1	2558	Berberidaceae	Ranales
145.	2539	Ranunculaceae	Ranales
165.7	2852	Papaveraceae	Rhocadales
167.9	3177	Saxifragaceae	Rosales

Volatile oils

Specific gravity. In table 3 are listed the average specific gravities of the volatile oils produced by tropical and temperate plant families. The plant families have been separated according to their respective climatic habitats, and from an inspection of the data it is evident that the volatile oils of tropical plant families have lower specific gravities than those produced by temperate plants (McNair, 1932).

In figure 4 the data from the tropical families of table 3 have been arranged in a scatter diagram. Each point indicates the average specific gravity of the volatile oil produced by a certain family shown by its numerical position in the Engler and Prantl system. A vertical line connecting various points shows the families of an order.

To find out whether the families higher on the evolutionary scale produce volatile oils of higher specific gravity, the trend is determined as a straight line extending from left to right on the diagram as in the previous cases of alkaloids (fig. 1) and glycerides (fig. 3).

It is assumed that the botanical numbers are free from any error, and therefore they are used as independent variables.

The equation formed by the method of least squares is

$$y = 0.90588 + 0.000002278x$$

where x indicates the botanical numbers and y the average specific gravities of the volatile oils (table 3). This equation has a positive slope ($+0.000002278$) which shows the line to have an upward inclination.

If both the botanical numbers and the specific gravities are considered as not free from error, the trend would have a similar direction.

It can therefore be definitely concluded that the higher the tropical plant family is in evolutionary development the greater will be its tendency to produce volatile oils of high specific gravity.

It can likewise be inferred (McNair, 1932) that terpenes and compounds of the fatty series predominate in the volatile oils produced lowest in the evolutionary position, while volatile oils formed by the families highest in evolution contain more aromatic, sulfur, and nitrogen compounds.

TABLE 3. *Volatile oils—their specific gravities and botanical and climatic distribution*

Sp. gr. at 15° (Gildemeister) y	Botanical no. (DeDalla Torre and Harms) x	Family (Engler and Prantl)	Order (Engler and Prantl)
Tropical			
.823	667	Palmae	Principes
.8526	1974	Urticaceae	Urticales
.8700	4543	Anacardiaceae	Sapindales
.8703	5162	Guttiferae	Parietales
.8722	3253	Pittosporaceae	Rosales
.874	1973	Moraceae	Urticales
.8741	6792	Asclepiadaceae	Contortae
.881	4916	Vitaceae	Rhamnales
.895	2737	Myristicaceae	Ranales
.8978	2665	Anonaceae	Ranales
.906	6968	Convolvulaceae	Tubiflorae
.9061	4140	Burseraceae	Geraniales
.9075	5090	Sterculiaceae	Malvales
.9136	5211	Dipterocarpaceae	Parietales
.917	4734	Sapindaceae	Sapindales
.9199	8381	Rubiaceae	Rubiales
.9213	4186	Meliaceae	Geraniales
.9219	7178	Verbenaceae	Tubiflorae
.9225	1324	Zingiberaceae	Scitamineae
.927	5254	Winteranaceae	Parietales
.9307	2567	Menispermaceae	Ranales
.9388	5558	Myrtaceae	Myrtiflorae
.948	6549	Apocynaceae	Contortae
.9482	2753	Monimiaceae	Ranales
.956	5355	Turneraceae	Parietales
.9565	2782	Lauraceae	Ranales
.964	684	Araceae	Spathiflorae
.9641	3980	Zygophyllaceae	Geraniales
.9648	7906	Acanthaceae	Tubiflorae
.9656	1859	Piperaceae	Piperales
.9667	2829	Hernandaceae	Ranales
Temperate			
.8741	3177	Saxifragaceae	Rosales
.8831	43	Pinaceae	Cephalataxaceae
.897	1872	Salicaceae	Salicales
.8998	8515	Caprifoliaceae	Rubiales
.9343	6063	Umbelliferae	Umbelliflorae
.9347	2539	Ranunculaceae	Ranales
.9590	5242	Cistaceae	Parietales
.9891	2961	Cruciferae	Rhoeadales
1.034	3122	Resedaceae	Rhoeadales
1.0772	1883	Betulaceae	Fagales

Refractive index. The refractive index is the ratio of the velocity of light in the oil compared with its velocity in air under the same conditions.

It is another property which may be used to measure variations in composition of volatile oils. A high refractive value may denote a small number of double bonds or a large quantity of compounds of high molecular weight. From table 4 it is evident that tropical volatile oils have higher values than temperate (McNair, 1932).

TABLE 4. *Volatile oils—their refractive indices and botanical and climatic distribution*

Refractive index at 20° (Gildemeister) y	Botanical no. (DeDalla Torre and Harms) x	Family (Engler and Prantl)	Order (Engler and Prantl)
Tropical			
1.4261	667	Palmae	Principes
1.4295	4916	Vitaceae	Rhamnales
1.4688	7906	Acanthaceae	Tubiflorae
1.4728	5090	Sterculiaceae	Malvales
1.4744	4543	Anacardiaceae	Sapindales
1.4835	1874	Myristicaceae	Ranales
1.4843	2665	Anonaceae	Ranales
1.4877	4140	Burseraceae	Geraniales
1.4883	1973	Moraceae	Urticales
1.4904	1324	Zingiberaceae	Scitamineae
1.4930	8381	Rubiaceae	Rubiales
1.4942	7178	Verbenaceae	Tubiflorae
1.5000	5211	Dipterocarpaceae	Parietales
1.5008	2829	Hernandiaceae	Ranales
1.5015	1859	Piperaceae	Piperales
1.5035	3980	Zygophyllaceae	Geraniales
1.5038	4186	Meliaceae	Geraniales
1.5053	684	Araceae	Spathiflorae
1.5184	2782	Lauraceae	Ranales
Temperate			
1.4760	6063	Umbelliferae	Umbelliflorae
1.4858	3177	Saxifragaceae	Rosales
1.4873	43	Pinaceae	Cephalotaxaceae
1.4922	1882	Juglandaceae	Juglandales
1.5099	2530	Ranunculaceae	Ranales
1.5274	2961	Cruciferae	Rhoeadales

In figure 5 the data from the tropical families of table 4 have been arranged in a scatter diagram. Each point indicates the average refractive index of the volatile oil produced by a certain family shown by its numerical position in the Engler and Prantl system. A vertical line connecting various points shows the families of an order.

To find out whether the families higher on the evolutionary scale produce volatile oils of lower refractive index, the trend is determined as a straight line extending from left to right on the diagram as in the previous cases of alkaloids (fig. 1), glycerides (fig. 3), and the specific gravities of volatile oils (fig. 4).

It is assumed that the botanical numbers are free from any error, and therefore they are used as independent variables.

The equation computed by the method of least squares is

$$y = 0.485705 - 0.000000028998x$$

where x indicates the botanical numbers and y the average refractive indices of the volatile oils. This equation has a negative slope ($-0.000000028998x$) which shows the line to have a downward trend.

If both the botanical numbers and the refractive indices are considered as not free from error, the trend would have a similar direction.

It can therefore be definitely concluded that the higher the tropical plant family is in evolutionary development, the smaller will be the average refractive index of its volatile oil.

It can likewise be inferred (McNair, 1932) that a smaller number or lesser amounts of saturated substances are found in the volatile oils produced lowest in the evolutionary position. A high refractive index may also indicate a large quantity of compounds of high molecular weight; therefore it might be that the volatile oils produced lowest in the evolutionary scale have less of these compounds.

However, it has been observed in volatile oils (McNair, 1932) that a low index of refraction carries with it a concomitant increase in specific gravity. Consequently, as the trend is downward in the case of the refractive index (fig. 5) and upward in the case of specific gravity (fig. 4), the values check in the case of evolutionary progression as well as in climatic difference.

It can therefore be concluded that the volatile oils of the tropical families highest in evolutionary development have constituents with a large number of double bonds (low saturation), more aromatic compounds, or more sulphur and nitrogen compounds with small amounts of substances of low molecular weight or small quantities of terpenes or bodies of the fatty series.

TROPICAL ACIDS

It has been shown from a consideration of both the specific gravity and refractive index of volatile oils that the higher the development of a tropical plant family the greater is the complexity of its chemical constituents. The study can likewise be continued to the various components of volatile oils—e.g., their acids and alcohols. An endeavor will be made to show that the heats of combustion of the alcohols and acids of tropical essential oils increase in harmony with the increase in evolutionary differentiation of the plant families producing them.

The relation between the heats of combustion (Kharasch, 1929) of the acids contained in essential oils and the degree of evolution of the plant families producing them (table 5, figure 6) is determined as follows:

Formic acid. Formic acid has been found in the essential oils of the following families (Gildemeister, 1922): (tropical) Burseraceae 4140, Myristicaceae 2737, Pittosporaceae 3252, Verbenaceae 7178, and Lauraceae 2782; (temperate) Umbelliferae 6063; (widely distributed) Compositae 8730,

Labiatae 7210, and Valerianaceae 8527. As the majority of these families are tropical, the substance formic acid is considered as mainly tropical.

TABLE 5. *Tropical acids of essential oils*

Acid	Heat of combustion (Kg. cal.)	Average botanical no.	Chemical formula	Chemical series
Formic	63	5624	HCOOH	Methane
Acetic	209	4328	CH ₃ COOH	Methane
n-Butyric	520	4668	CH ₃ (CH ₂) ₂ COOH	Methane
n-Valeric	679	5171	CH ₃ (CH ₂) ₃ COOH	Methane
n-Caproic	831	5032	C ₆ H ₁₁ COOH	Methane
Benzoic	711	2843	C ₆ H ₅ COOH	Aromatic
Cinnamic	1040	3566	C ₆ H ₅ .CH:CH.COOH	Aromatic
Cuminic	1238	2782*	(CH ₃) ₂ CH.C ₆ H ₁₁ .COOH	Aromatic
Capric	1453	4908	CH ₃ (CH ₂) ₈ COOH	Methane
Palmitic	2380	4214	C ₁₅ H ₃₁ COOH	Methane

* Observed in only one family.

The numbers following the family names in the above list are the serial numbers used by DeDalla Torre and Harms (1907) in their list of angio-

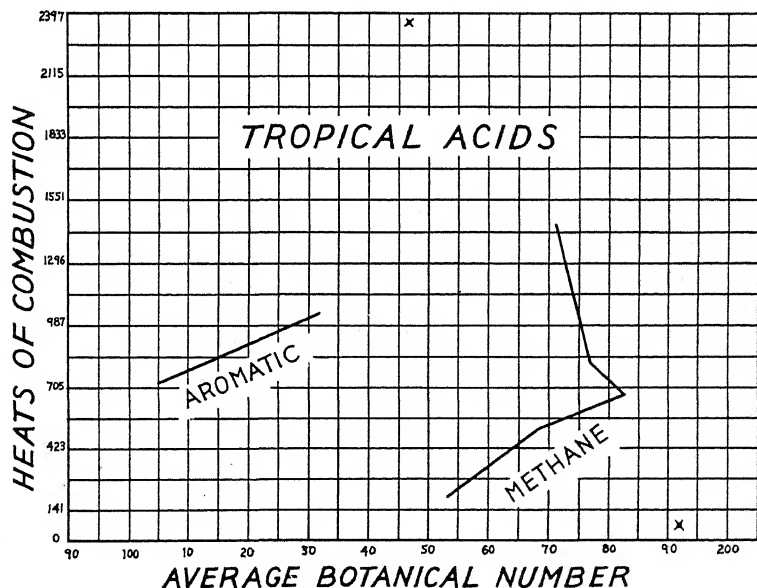


Fig. 6

sperms according to the Engler system of classification. The sum of these numbers divided by the number of the families ($50619 \div 9$) is the average serial number for formic acid. This quotient, 5624, consequently represents the position of formic acid in the scale of plant evolution.

Acetic acid. Acetic acid is known to occur in the essential oils of the following families (Gildemeister, 1922): (tropical) Anonaceae 2665, Bur-

seraceae 4140, Myristicaceae 2737, Rubiaceae 8381, Sterculiaceae 5090, Zingiberaceae 1324, Myrtaceae 5558, and Lauraceae 2782; (tropical-subtropical) Geraniaceae 3924, and Magnoliaceae 2651; (subtropical) Hamamelidaceae 3295; (temperate) Pinaceae gymnosperm, Umbelliferae 6063, Cruciferae 2961, and Polygonaceae 2184; and (widely distributed) Valerianaceae 8527, Aristolochiaceae 2169, Rutaceae 3986, Gramineae 101, Oleaceae 6419, Compositae 8730, and Labiatae 7210. As the majority of these families are tropical, their product, acetic acid, is likewise considered as tropical. The sum of their appended serial numbers is 90897; dividing by the number of families, 21, gives 4328 as quotient, which represents the average position in evolution of the families producing acetic acid.

Butyric acid. Butyric acid is known to occur in the essential oils of these families (Gildemeister): (tropical) Lauraceae 2782, Myristicaceae 2737, Myrtaceae 5558, Sterculiaceae 5090, and Verbenaceae 7178; (tropical-subtropical) Geraniaceae 3924; (temperate) Polygonaceae 2184, and Umbelliferae 6063; (widely distributed) Gramineae 101, Labiatae 7210, and Valerianaceae 8527. As most of these families are tropical, their product, butyric acid, is likewise considered as tropical. The sum of their appended serial numbers is 51354; dividing by the number of families, 11, gives 4668 as quotient, which represents the average evolutionary position of the plant families that produce butyric acid.

Valeric acid. Valeric acid has been found in the essential oils of the following families (Gildemeister): (tropical) Anonaceae 2667, Lauraceae 2782, Myrtaceae 5558, Pittosporaceae 3252, and Sterculiaceae 5090; (tropical-subtropical) Geraniaceae 3924; (temperate) Umbelliferae 6063; (widely distributed) Compositae 8730, Labiatae 7210, Polygalaceae 4273, Rutaceae 3986, and Valerianaceae 8527. Most of the above families are tropical; consequently their product, valeric acid, is likewise considered as tropical. Their serial numbers attached add up to 62060. This divided by the number of families, 12, gives the quotient of 5171, which may be considered as the average position in evolution of the plant families producing valeric acid.

Caproic acid. Caproic (capronic) acid occurs in the essential oils of the following families (Gildemeister): (tropical) Lauraceae 2782, Palmae 667, Rubiaceae 8381, and Sterculiaceae 5090; (temperate) Pinaceae gymnosperm, and Umbelliferae 6063; (widely distributed) Labiatae 7210. As the majority of these families are tropical, their product, caproic acid, is likewise considered as tropical. The sum of their appended serial numbers is 30193; dividing by the number of families, 6, gives 5032 as quotient, which represents the average position in evolution of the plant families producing caproic acid.

Benzoic acid. Benzoic acid, according to Gildemeister (1922), is found in the essential oils of the following families: (tropical) Anonaceae 2665, Lauraceae 2782, and Myrtaceae 5558; (tropical-subtropical) Amaryllidaceae 1166; (widely distributed) Chenopodiaceae 2214, Leguminosae 3435, Liliaceae 942, and Rutaceae 3986. As the majority of these families are tropical, their

product, benzoic acid, is likewise considered as tropical. The sum of their appended serial numbers is 22748; dividing by the number of families, 8, gives 2843 as quotient, which represents the average position in evolution of the plant families producing benzoic acid.

Cinnamic acid. Cinnamic acid, according to Gildemeister (1922), is found in the essential oils of the following families: (tropical) Lauraceae 2782, Myrtaceae 5558, and Zingiberaceae 1324; (subtropical) Hamamelidaceae 3295; (widely distributed) Labiatae 7210, Leguminosae 3435, Liliaceae 942, and Rutaceae 3986. As most of these families are tropical, their product, cinnamic acid, is likewise considered as tropical. The sum of their appended serial numbers is 28532; dividing by the number of families, 8, gives 3566 as quotient, which represents the average position in evolution of the plant families producing cinnamic acid.

Cuminic (cunic) acid. Cuminic acid is found in the volatile oils of but one family, according to Gildemeister (1922). The family is a tropical one, the Lauraceae, with a botanical serial number (DeDalla Torre and Harms, 1907) of 2782.

Caprinic (capric) acid. Caprinic acid has been found in the volatile oils of six families (Gildemeister, 1922); they are: (tropical) Palmae 667, Sterculiaceae 5090, and Vitaceae 4916; (temperate) Umbelliferae 6063; and (widely distributed) Compositae 8730 and Rutaceae 3986. Most of these families are tropical in distribution; consequently caprinic acid produced by them is tropical. As the sum of their appended serial numbers is 29452, which when divided by the number of families, 6, gives a quotient of 4908, the average position in evolution of the plant families which form caprinic acid is 4908.

Palmitic acid. Palmitic acid, which is familiar as a constituent of fatty oils, has also been found in many volatile oils. Gildemeister's (1922) summary furnishes the following: (tropical) Anacardiaceae 4543, Anonaceae 2665, Araceae 684, Asclepiadaceae 6792, Burseraceae 4140, Lauraceae 2782, Myrtaceae 5558, Palmae 667, Piperaceae 1859, Pittosporaceae 3252, and Verbenaceae 7178; (subtropical) Myricaceae 1874; (temperate) Betulaceae 1887, Caprifoliaceae 8515, Ranunculaceae 2539, and Umbelliferae 6063; (widely distributed) Aristolochiaceae 2169, Compositae 8730, Euphorbiaceae 4286, Labiatae 7210, Leguminosae 3435, Liliaceae 942, Malvaceae 4980, Rutaceae 3986, and Valerianaceae 8527. These families are mostly tropical in habitat, and as a consequence the substance found in them (palmitic acid) may be considered as mostly tropical. The serial numbers appended to the families sum up to 105363. As the total number of families is 25, the average serial number is $\frac{105363}{25} = 4214$. The position of these families in plant evolution may therefore be taken as 4214.

When the average botanical numbers for the families that produce the various acids are used as one coordinate and the heats of combustion of their

corresponding acids are used as the other coordinate, upward curves are obtained. It is required to treat separately the aromatic and methane series of acids, each of which forms an upward-sloping curve. From a consideration of these curves the tendency is evident for the acids of greater heats of combustion to be formed by plant families higher in the scale of evolution.

TABLE 6. *Tropical alcohols of essential oils*

Alcohol	Heat of combustion (Kg. cal.)	Average botanical no.	Chemical formula	Chemical series
Ethyl	328	4619	C_2H_5OH	Methane
Isobutyl	638	5558	$(CH_3)_2CH.CH_2OH$	Methane
n-Amyl	792	6603	$CH_3(CH_2)_4CH_2OH$	Methane
n-Heptyl	1104	5558	$(CH_3)_2(CH_2)_4CHOH$	Methane
Borneol	1465	4192	$C_{10}H_{17}OH$	Hydro-aromatic
Terpineol	1475	4801	$C_{10}H_{17}OH$	Hydro-aromatic

TROPICAL ALCOHOLS

In the tropical essential oils (table 6; fig. 7) that have been analyzed (Gildemeister, 1922), six alcohols are known whose heats of combustion have been determined (Kharasch, 1929). Four of these belong to the methane

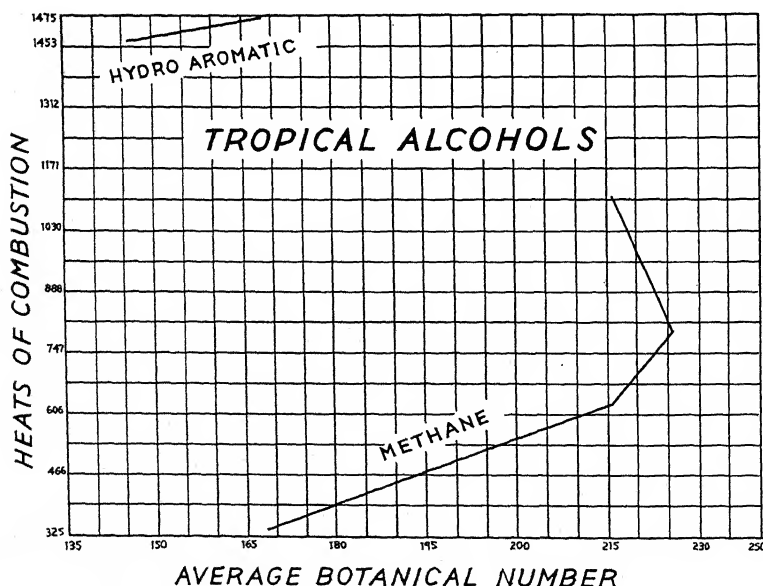


Fig. 7

or saturated series—namely, ethyl, isobutyl, normal amyl, and normal heptyl alcohols. Two of them are of the alicyclic or hydro-aromatic series—namely, borneol and terpineol.

Ethyl alcohol. Ethyl alcohol has been located in the essential oils of eight families, according to statistics compiled by Gildemeister (1922). These families are: (tropical) Myrtaceae 5558, Rubiaceae 8381, Vitaceae 4916, and Zingiberaceae 1324; (subtropical) Hamamelidaceae 3295; (temperate) Umbelliferae 6063, and (widely distributed) Leguminosae 3435 and Rutaceae 3986. The above families are tropical for the most part, and for that reason ethyl alcohol which is made by them is considered as mostly tropical in location of formation. The serial numbers which follow the names of the families add up to 36958. As there are eight families, the average serial number is $36958 \div 8$, or 4619.

Isobutyl alcohol. Isobutyl alcohol has been located in the essential oils of only one family, the Myrtaceae 5558. As this family is mainly tropical in habitat, isobutyl alcohol is likewise classed as tropical, with a botanical serial number of 5558.

Amyl alcohol. Amyl alcohol is found in the essential oils of four families (Gildemeister, 1922). These are: (tropical) Myrtaceae 5558 and Vitaceae 4816; and (widely distributed) Compositae 8730 and Labiatae 7210. The sum of the serial numbers (DeDalla Torre and Harms, 1907) of these families is 26414, with an average of 6603.

Heptyl alcohol. Normal heptyl alcohol is found, according to Gildemeister (1922), in but one family, the Myrtaceae, with a serial number of 5558. As this family is tropical, the substance is placed among those formed in the tropics.

Borneol. Borneol has been separated from the essential oils of the following families (Gildemeister, 1922): (tropical) Anacardiaceae 4543, Burseraceae 4140, Dipterocarpaceae 5211, Lauraceae 2782, Myristicaceae 2737, Piperaceae 1859, and Zingiberaceae 1324; (subtropical) Hamamelidaceae 3295; (temperate) Pinaceae gymnosperm, Umbelliferae 6063, and (widely distributed) Aristolochiaceae 2169, Compositae 8730, Gramineae 101, Labiatae 7210, and Valerianaceae 8527. Most of these families are tropical in habitat and for this reason borneol is listed as mainly tropical. The sum of the serial numbers of these families is 58691; this divided by the sum of the families, 14, gives the average serial number 4192 as the quotient.

Terpineol. Terpineol, which likewise occurs in essential oils, has been isolated from those of the following families (Gildemeister, 1922): (tropical) Burseraceae 4140, Dipterocarpaceae 5211, Lauraceae 2782, Myristicaceae 2737, Myrtaceae 5558, Rubiaceae 8381, and Zingiberaceae 1324; (tropical-subtropical) Geraniaceae 3924 and Magnoliaceae 2651; (temperate) Pinaceae gymnosperm, and Umbelliferae 6063; (widely distributed) Aristolochiaceae 2169, Compositae 8730, Labiatae 7210, Leguminosae 3435, Rutaceae 3986, and Valerianaceae 8527. The total of the serial numbers (DeDalla Torre and Harms, 1907) of these families is 76828. As there are 16 families, the average serial number is 4801.

When the heats of combustion of these tropical alcohols are treated as

two groups—the hydro-aromatic and the methane alcohols—and used as coordinates in conjunction with the average botanical numbers of the families which make them, two curves are formed. Both of these curves have upward inclinations and consequently designate the families with higher average botanical numbers as more likely to produce alcohols of higher heats of combustion.

SUMMARY

The object of this paper is to show that, when the plant families which contain fats, volatile oils, and alkaloids are first separated according to the climate of habitat, some chemical and physical properties of these substances vary in accordance with the degree of evolution of the plant families containing them; and the probability is that, the more highly organized the plant, the more complex are its chemical products.

The fact that climatic changes in physical and chemical properties from glycerides, volatile oils, and alkaloids are consistent causes one to deduce that a sufficient number of chemical analyses of the determinant substances have been made from sufficiently representative ranges of evenly dispersed families and that any additional analyses will fall in the groups already established and will only more strongly confirm the conclusion already determined.

In any one climate any physical or chemical changes in specific chemical products may perhaps serve as an index of the degree in evolution of the plant family in which these changes took place.

It is necessary that the chemical substances used for this purpose be as specific as the plant unit used, be it family, genus, or species.

Glycerides, volatile oils, and alkaloids are defined and shown to have certain chemical properties often specific for species.

Some constituents of volatile oils have already been used to show morphological evolution in the genera of *Eucalyptus* and *Callitris*. Alkaloids have been used in the genus *Aconitum*.

Tropical alkaloids may be arranged in a scatter diagram with the average molecular weights and the botanical position of the containing plant families as coordinates. When this is done, the straight-line trend produced indicates that the higher the plant family in evolutionary progression the more likely it is to produce alkaloids of high molecular weight.

Likewise it is shown that alkaloids which are formed by more than one family when first separated as to climate assume positions in the evolutionary scale proportional to their average molecular weights.

Similarly, tropical glycerides may be arranged in a scatter diagram in which the average iodine numbers and the botanical positions of the producing plant families are used as coordinates. When this is done the straight-line trend produced indicates that the higher the plant family in evolutionary position the more likely it is to form glycerides with large iodine numbers (and low melting points).

In the case of tropical volatile oils scatter diagrams may be made with their specific gravities as one coordinate and the botanical positions of the producing plant families as the other coordinate. The resulting trend is upward in accordance with the increase in specific gravity and the increase in plant evolution.

Another scatter diagram for tropical volatile oils may be made in which one coordinate is the refractive index and the other is the producing family's evolutionary position. The trend of this arrangement is down and coincides with the results obtained from the use of the specific gravity, for a low index in refraction carries with it a concomitant increase in specific gravity.

It can therefore be concluded that the volatile oils of the families highest in evolutionary development have constituents with a large number of double bonds (low saturation), more aromatic compounds, or more sulfur and nitrogen compounds with small amounts of substances of low molecular weight or small quantities of terpenes or bodies of the fatty series.

The heat of combustion of the acids contained in the tropical volatile oils increases with the increase in the average systematic position of the families producing them. This coincides with the findings obtained from the study of the specific gravity and refractive index of volatile oils.

The heats of combustion of the alcohols which are constituents of the tropical volatile oils increase with the increase in the average systematic position of the families producing them. This result likewise agrees with and confirms the findings obtained from the study of the specific gravities and refractive indices of volatile oils.

All of these results demonstrate what may be a general rule for the tropics and perhaps for all climates—that the more highly organized the plant the more highly organized are likely to be its chemical products.

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THE MORPHOLOGY OF THE FLOWERS OF *ROSA* AND CERTAIN CLOSELY RELATED GENERA

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The morphology of the flowers of the Rosaceae has long been a subject of discussion. The family shows wide variation in flower type, but the ordinary rosaceous flowers (excluding the zygomorphic group) may be placed in two general groups: in the first, those in which the gynoecium is wholly free from other parts of the flower (ovary superior); in the second, those in which the gynoecium is not entirely free (ovary inferior or partly so). In this second group belong a number of rosaceous flowers in which the gynoecium, though on external examination apparently free, is found on sectioning to be partially fused with other parts.

A discussion of the morphology of the flower types of the entire family would, if presented in a single paper, run far beyond desirable limits. The present paper concerns certain rosaceous flowers of the first group only.

In rosaceous flowers of this type the sepals, petals, and stamens arise on the margin of a tube-like, disc-like, or cup-like portion of the flower which surrounds, to varying extent, the gynoecium. This tube-like part of this type of flower has been designated by various names. In the present paper it is referred to as the tube or the tube-like part of the flower because these two terms imply nothing as to its inherent morphology. It is the presence of this tube which has caused this type of flower to be the subject of much investigation and discussion.

The explanations offered by investigators of the subject and the statements made by others concerning the nature of the tube in this type of rosaceous flower fall into three general groups: statements and explanations of the tube as an axial structure; statements and explanations of the tube as a structure formed of floral appendages and therefore appendicular in morphological nature; explanations in which a distinction is drawn between the rose flower and those of other rosaceous flowers with superior gynoecium with reference to the morphological nature of the tube.

The present investigation was undertaken to see if information might be obtained from a comparative study of the vascular structure of the flower of the rose and that of some closely related types which would aid in establishing the morphology of the tube-like part of those flowers. During the progress of the work there was published by Bonne (1928) a paper on the Rosaceae which presents the vascular structure of a great number of rosaceous flowers, including those of the genus *Rosa*. Bonne considers the tube of rosaceous

flowers, other than *Rosa*, as appendicular. The tube of the rose flower she considers to be partly axial, partly appendicular. Her interpretations are in agreement with those arrived at by the author from the present study except with respect to certain minor details.

The work of Bonne has not, however, come to be generally known, undoubtedly on account of its method of publication (published privately in France). Nor has the work of earlier authors who hold the same opinion as Bonne come to be generally known or accepted. Textbooks and most other botanical works still present the axial theory. It has, therefore, seemed to the author desirable to offer her evidence and her interpretation of that evidence and thus make generally available the observed anatomical data and an interpretation of the tube of rosaceous flowers based upon those data.

A study of the vascular structure of several rosaceous flowers (with superior gynoecium) such as *Dalibarda repens* L., *Rubus triflorus* Richards, *Dryas integrifolia* Vahl., etc., and a comparison of their structure with that of a simple hypogynous flower such as that of the buttercup provide evidence for an intelligible and logical explanation of the nature of the tube-like part of these flowers.

In a simple hypogynous flower the receptacular stele gives rise to traces to the sepals, petals, and stamens in that order (fig. 1). After the bundles to the sepals, petals, and stamens have left the stele, the remaining stelar tissue continues upward through the elongated receptacle and gives rise to the carpel traces. It is obvious that this vascular tissue is the direct continuation of the receptacular stele lower down and that the carpel traces are the last traces to floral organs to which it gives rise.

In a simple rosaceous flower such as that of *Dalibarda repens*, the receptacular stele gives rise to bundles which supply sepals, petals, stamens, and carpels. These bundles arise in that order as in the buttercup (fig. 2). Due to vertical compression of the receptacle (in phylogeny), these bundles arise much closer together than in the buttercup flower. The bundles which supply the sepals, petals, and stamens pass out from the receptacular stele, run upward through a short tube, and then pass into the free bases of those organs. The stelar tissue which does not pass out to supply the sepals, petals, and stamens continues upward through the receptacle and gives rise to carpel traces. Most of the bundles which supply stamens split into two or more strands, each of which runs into a free stamen base (right side of fig. 2). The stamens are more numerous than the perianth parts and there are stamens which lie in radii in between the sepal and petal radii. The bundles of these stamens arise from the stele in the same way as do those of the other stamens and behave as those do.

The vascular structure of the flower of *Rubus triflorus* is essentially the same as that of *Dalibarda*. The tube is, however, much longer.

In *Dryas integrifolia* the receptacular stele gives rise to a ring of bundles which pass outward and upward through the tube. The remaining stelar

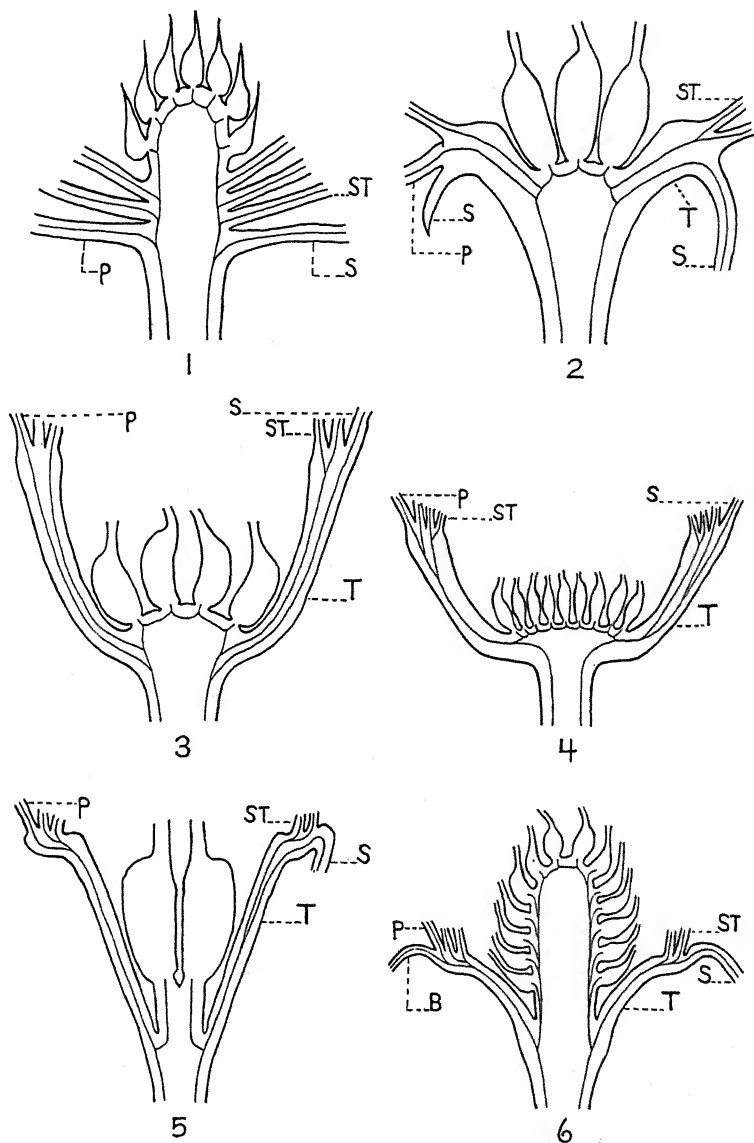


Fig. 1-6. Fig. 1, longitudinal diagram of the flower of *Ranunculus fascicularis* in a plane through the median radius of a sepal and the opposite petal. Fig. 2-6, longitudinal diagrams of five rosaceous flowers in a plane through the median radius of a sepal and the opposite petal: fig. 2, *Dalibarda repens*; fig. 3, *Rubus triflorus*; fig. 4, *Dryas integrifolia*; fig. 5, *Waldsteinia fragarioides*; fig. 6, *Geum strictum*. S., sepal; P., petal; ST., stamen; B., bract; T., tube.

tissue continues up through the receptacular tissue and becomes carpel traces (fig. 4). The ring of bundles which pass up through the tube supplies sepals, petals, and stamens. Most of these bundles lie in the median radii of the perianth parts (*Dryas* has 8-9 sepals and 8-9 petals). A few of these bundles lie in radii between the median radii of sepals and petals. These latter supply stamens only and commonly break into two or more strands, each of which passes into a free stamen base. Each of the bundles in the radii of the perianth parts supplies a sepal or petal and some stamens. The bundles to most of the stamens are derived thus. These stamens separate from the main bundles at various levels and pass upward through the tube into the free stamen bases. Commonly several such bundles separate from each of the main bundles. These may come off at one level or at different levels. After their separation from the main bundles, these stamen bundles usually split into two or more strands, each of which passes into a free stamen base.

In the flower of *Waldsteinia fragarioides* (Michx.) Tratt. (fig. 5) ten bundles pass outward from the stele of the receptacle into the tube. The remaining stelar tissue moves inward and upward and gives rise to carpel traces. The ten main bundles of the tube supply sepals, petals, and all of the stamens. The bundles to the stamens become separate in the great majority of cases at a high level in the tube—a short distance below the free bases of the stamens. The left side of figure 5 shows this common condition. Some of the stamen bundles become free from the ten main bundles lower down in the tube—about halfway down. The right side of figure 5 shows this condition. After their separation from the main bundles the stamen bundles commonly split into two or more strands, each of which runs into a free stamen base.

The vascular structure of *Geum strictum* Ait. (fig. 6) is essentially like that of *Waldsteinia*. It differs in two respects which are of importance. All the stamen bundles are derived from the ten main bundles at a high level—just below the free bases of the stamens. There are present, in *Geum*, bracts outside of and alternate with the sepals. The bundles to these bracts separate from the five main bundles which lie in the same radii as the bracts, that is, in the petal radii. This separation takes place just below the insertion of the bracts.

By comparing the figure of the flower of *Dalibarda* with that of the buttercup (fig. 2, 3), it is readily seen that the tube-like part of the *Dalibarda* flower could easily be formed artificially by compressing the buttercup flower vertically and uniting the bases of the sepals, petals, and stamens for a short distance. From the evidence of the vascular anatomy, this is clearly what the tube of *Dalibarda* really represents—merely the united basal portions of the sepals, petals, and stamens. The receptacle to which these organs are attached has become vertically shortened, and the bases of the crowded appendages have become fused. Fusion has not, however, extended to the traces of these organs which run through the tube free from one another

except in the case of the stamen traces, most of which have become united with other stamen traces. This union of some of the traces of the numerous stamens would naturally result from the vertical shortening of the floral axis. Aside from this fusion of stamen traces with one another, the vascular plan of the flower of *Dalibarda* differs in no essential respect from that of the buttercup flower. The flower is merely much compressed vertically, and the traces to the floral organs come off much closer together because of that compression.

The vascular structure of the flower of *Rubus triflorus* is essentially like that of *Dalibarda*. The tube-like part is of the same nature; it is merely much longer than in *Dalibarda*.

Dryas shows a somewhat more complicated condition in that the stamen bundles have become united with those of the perianth parts in most cases. The bundles which run through the tube of *Dryas* are not, therefore, simple traces to floral parts but are groups of traces fused. This fusion is brought about by increased shortening of the distance between the levels of origin from the stele, of the traces to the perianth parts and the stamens. Stamen bundles lying in the same radii, or very nearly in the same radii, as the perianth traces are thus brought very close to those traces and become fused to them. Other stamen bundles may remain free. In *Dryas* there are a few such stamen bundles. These pass out from the stele with the perianth (and fused stamen bundles) but are free from those bundles throughout the tube. The union of stamen bundles with bundles to the perianth parts is, in *Dryas*, irregular in vertical extent; the stamen bundles become free at varying heights.

In *Waldsteinia* and *Geum* further stages in progress of this fusion are seen. In *Waldsteinia* most of the stamen bundles are united with perianth bundles throughout practically the entire length of the tube. A few are united for a shorter distance. There are in *Waldsteinia* no stamen bundles in radii between the sepal and petal radii. The bundles of the stamens which lie in those radii have become fused with the perianth bundles. In *Geum* all of the stamen bundles are united with the perianth bundles throughout practically the entire length of the tube. The bundles of the bracts are also united with bundles in the radii in which they lie throughout the length of the tube.

It seems clear from this comparative examination that the tube of these rosaceous flowers is formed of the fused basal portions of the sepals, petals, and stamens (and in *Geum* of the bracts also). Furthermore, it seems evident that, in some cases, the external fusion of these floral parts has been followed by internal fusion—fusion of the traces of these organs. This vascular fusion shows various stages of completeness in the forms described. It is most highly developed in the flower of *Geum*. The free sepals, petals, and stamens (and, in *Geum*, bracts) which appear to arise on the margin of the tube are merely the free upper portions of these organs and do not represent entire organs. These organs are not inserted on the tube as they appear to be but on the receptacle as in an ordinary hypogynous flower.

The tube-like portion of other rosaceous flowers with free gynoecium is of the same nature as in the flowers described except in the case of *Rosa* (and *Nuttallia*, *Prinsepia*, and *Chamaebatia* examined by Bonne but not by the present writer).

Let us examine now the vascular structure of a rose flower to see what evidence it presents as to the morphological nature of the tube-like part of that flower.

In the flower of *Rosa Helenae* Rehder and Wilson, five large bundles move gradually outward from the widened receptacular stele in the radii of the

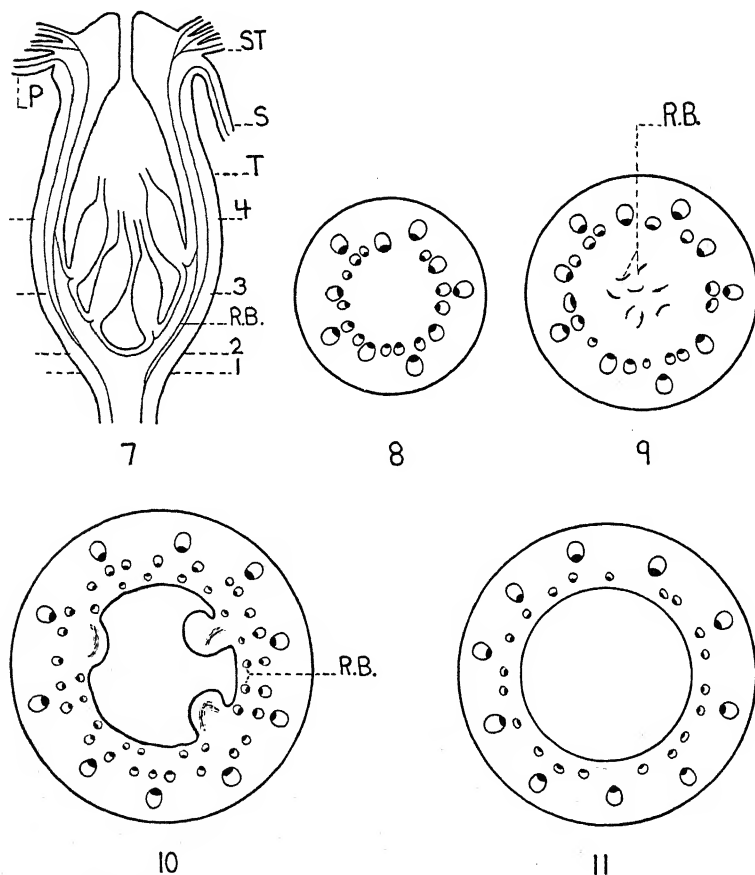


Fig. 7-11. *Rosa Helenae*. Fig. 7, longitudinal diagram of the flower in a plane through the median radius of a sepal and the opposite petal. Fig. 8-11, cross-section diagrams of the flower at successive levels. These levels are indicated on figure 7 by lines 1-4. R. B., recurrent bundles; T., tube.

sepals (fig. 8). A section somewhat farther up shows five more strong bundles, in the petal radii, which have become differentiated and which are

passing gradually out of the stelar circle (fig. 9). At this level (level 2 of fig. 7) there appear in the centre of the flower a number of small strands, marked R. B. in figure 9. These strands are not connected with the receptacular vascular tissue at this level. They are recurrent, or down-branched, strands which run along the inner margin of the tube and give rise to the carpel traces (fig. 7, 10). The five large bundles in the petal radii move gradually farther out of the stelar circle and come to lie in practically the same circle as those in the sepal radii. The remaining stelar tissue forms a ring of smaller bundles just within this sepal-petal whorl. Within this second whorl is the circle of down-branched strands which give rise at various levels to traces to the carpels (fig. 7, 10). There are, thus, at this level (level 3 of fig. 7) three circles of bundles, the innermost of which shows inverse orientation of the xylem and phloem elements (fig. 10). These inverted bundles become connected with the middle whorl of bundles at various levels from somewhat above the level of insertion of the lowest carpel to somewhat above the level of insertion of the highest carpel. A section taken above the level of the highest connection of these two whorls of bundles shows the sepal-petal whorl of large bundles and within this a whorl of small bundles (fig. 11, 7). The sepal-petal whorl supplies the sepals and petals. The inner whorl supplies the stamens. These stamen bundles branch freely in the upper part of the tube and the branches thus formed pass into the free stamen bases.

In a simple hypogynous flower such as that of the buttercup, the carpels are obviously the uppermost of the floral organs. In the case of the flowers of *Dalibarda*, *Rubus*, etc., we have shown that an apparent lower position of the carpels is brought about by modification of the flower—by the fusion of the bases of the outer floral parts into a tube which surrounds the carpel-bearing portion of the floral axis and which appears to bear on its margin the outer floral parts. In the rose flower a similar process has taken place and accounts to some extent for the low position of the carpels. That there is something further involved in the case of the rose is evidenced by the presence, in *Rosa*, of the inverted bundles which are not present in the rosaceous flowers described above.

A comparison of the rose flower with that of *Rubus triflorus* is helpful at this point (fig. 12, 13). If the receptacular portion of the *Rubus* flower which bears the carpels were invaginated and the bundles which run through it were thus inverted, a flower fundamentally similar to that of the rose would be produced. Figure 15 shows such a hypothetical flower in longitudinal aspect. In the rose flower the basal portion is much dilated and the flower appears, therefore, at first glance quite dissimilar to the hypothetical flower shown in figure 15. Careful comparison of figures 12 and 15 will, however, show their fundamental similarity.

It is evident from the vascular structure that such an invagination has taken place in the phylogenetic development of the rose flower and has brought about the inverted condition of the inner ring of bundles in *Rosa*.

These bundles are, in reality, the continuation of the receptacular stele of the pedicel and are homologous with the normally oriented stelar bundles in *Rubus* (and in the other forms described) which run up through the carpel-bearing portion of the receptacle and give rise to carpel traces. Comparison of figures 12, 13, and 15 will make this clear.

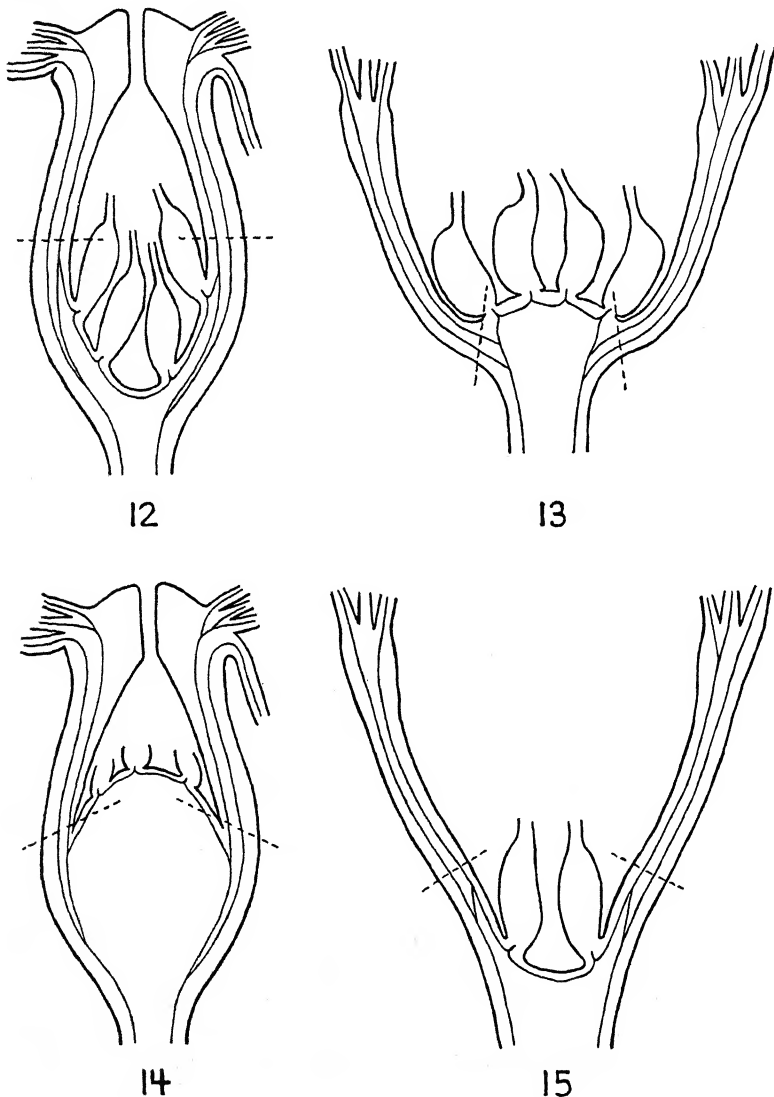


Fig. 12-15. Fig. 12, longitudinal diagram of the flower of *Rosa Helenae* in a plane through the median radius of a sepal and opposite petal. Fig. 13, longitudinal diagram of the flower of *Rubus triflorus* in a plane through the median radius of a sepal and opposite petal. Fig. 14-15, explanation in the text. The dotted lines mark the approximate limit between the axial and appendicular portions of the flowers.

The middle ring of bundles which runs up through the lower part of the rose tube and which connects with (really continues downward as) the inverted bundles (fig. 7, 10, 12) is composed of stelar bundles. These bundles are homologous with the stelar bundles in *Rubus triflorus* which continue upward between the level of origin of the lowest perianth bundles and that of the highest stamen bundles. Comparison of figures 12, 13, and 15 will show this.

Figure 14 shows a diagram in longitudinal aspect of a *Rosa Helenae* flower identical with figures 7 and 12 except that the carpel-bearing part is represented as convex instead of concave. The axis end is no longer invaginated, and the inverted bundles of the normal rose flower are, in the hypothetical flower shown in figure 14, not inverted. Comparison of this figure with figure 12 will show what has happened in the development (phylogenetically) of the rose flower.

The tube of the rose flower is, then, really partly axial, for through its lower portion run stelar bundles which give rise to traces to floral parts. This lower part of the tube is a hollow axis in which the morphological apex is at the base of the hollow as the result of invagination in phylogenetic development.

The upper portion of the tube of the rose flower is not axial in nature, for the stelar bundles do not continue up through all the extent of the tube. They go up only as far as the highest levels of their bending back and running down in the inner part of the tube. Through that part of the tube above this level run the ten large perianth bundles and the stamen bundles (fig. 7, 11). This part of the rose tube is morphologically strictly similar to the tube of rosaceous flowers such as *Dalibarda*, *Rubus*, etc. It is formed of the fused bases of the sepals, petals, and stamens. These organs are not inserted on the margin of the tube as they appear to be. On the contrary, their upper portions are non-united and these free upper portions appear as free organs.

The low position of the carpels in *Rosa* is brought about by a combination of this tube formation and the invagination of the apical part of the floral axis. The carpels are really the uppermost organs on the floral axis, as in all angiosperm flowers.

The exact limit between the axial and the appendicular portions of the rose flower cannot be determined. The approximate limit is at a level slightly above that of the highest point of bending back of the stelar bundles. This will be apparent from inspection of the diagrams of *Rubus* and *Rosa* (fig. 12, 13), in which the approximate limit between the axial and appendicular portions of these flowers is indicated. Those portions of these flowers distal to the dotted lines are appendicular. Those portions which are proximal to the dotted lines are receptacular or axial. As has been stated, the limit is only approximate in the case of the rose flower. This is because of the highly modified condition of this flower. Comparison of figures 13 and 15 and of figures 12 and 14 will help to show this.

In the course of this investigation the author has studied the vascular structure of a number of species of the genus *Rosa*. They are by no means identical in vascular plan, although fundamentally alike. The differences observed are not of value in the present discussion, and their inclusion here would only confuse matters. It seems well to state merely that the description of *Rosa Helenae* is not meant to be typical of all roses with respect to all features of the vascular plan.

In the descriptions of the other rosaceous forms given above certain minor vascular features are also omitted for the same reason.

It is not thought desirable to include in this paper a detailed account of all previous investigations and opinions on the morphology of the tube of rosaceous flowers of the type under discussion; it seems sufficient to touch briefly upon certain of these and to indicate the evidence, if any, upon which they are based. In this discussion of certain previous opinions and investigations, the word tube is used with the same meaning as in the first part of this paper in order to avoid confusion. This usage does not imply that all writers on the subject have so used the term.

Schleiden (1849), Sachs (1868), Baillon (1869), Le Maout and Decaisne (1876), Gray (1879), Foche (in Engler and Prantl), 1894, Strasburger (1912), Rendle (1925), Warming and Möbius (1929), Eichler (1878), Pool (1929), Johnson (1931), Smith et al. (1928), Rehder (1927), Hegi (1923), Robbins and Rickett (1929), and Torrey (1932) consider that the tube represents the dilated and hollow floral receptacle, but cite no investigations which furnish evidence in support of this opinion. Payer (1857) and Goebel (1887) present ontogenetic developmental evidence which, they say, demonstrates the receptacular nature of the tube. Le Maout and Decaisne (1876) state that the developmental evidence shows that the tube is receptacular. Vines (1895) and Pax (1890) say that intercalary growth of a portion of the axis gives rise to the tube. Rydberg (1898) considers that the tube is axial because the petals and stamens cannot be borne on the sepals; because the petals are deciduous at their points of insertion on the margin of the tube, and the points at which abscission takes place should represent the bases of these organs; and because the vascular bundles of the sepals, petals, and stamens are not free through the tube as they should be if the sepals, petals, and stamens were inserted at the base of the tube.

DeCandolle (1825), in the *Prodromus*, states with reference to the Rosaceae that the sepals are coherent into a calyx-tube on which the petals and stamens are inserted. In his *Cours de Botanique* he discusses this type of flower in more detail. The tube, as he describes it, consists of the united basal portions of the sepals, petals, and stamens. Lindley (1853), in *The Vegetable Kingdom*, says that the stamens arise from the calyx just within the petals. In his *Introduction to Botany* (1839), he says that when botanists describe the stamens as inserted on the calyx they are inaccurate, as the stamens are really coherent with the calyx up to a certain point. DeCandolle

and Lindley offer no evidence to support this view. Don (1832), Bentham and Hooker (1862-1867), Duchartre (1867), Hutchinson (1926), and Sinnott (1929) call the tube calyx. They offer no evidence to support their opinions. Velenovský (1913) states that the tube of the Rosaceae is merely a calyx structure. His opinion is based largely upon study of teratological specimens. Domin (1914), from a study of abnormal specimens, concludes that the tube consists of the stipule parts of the calyx leaves. The petals and stamens are in no way involved, he says. He considers that the anatomical structure does not furnish reliable evidence as to the nature of the tube.

Jussieu (1842) considers that the tube of *Rosa* is partly axial, partly appendicular. The basal part is receptacular, the upper part is calyx. The tube of other rosaceous flowers with free gynoecium he considers calyx. He presents no evidence. Van Tieghem (1875) studied the vascular anatomical structure of the tube and concludes that the vascular structure shows that the tube is appendicular and consists of the united basal portions of the sepals, petals, and stamens except in *Rosa*. In *Rosa* he considers the tube partly axial, partly appendicular. The tube of *Rosa* is axial up to the level of the highest bending back of stelar bundles. From that level on up, the tube is appendicular and is formed of the united basal portions of the sepals, petals, and stamens. Bonnier (1881), from an anatomical study of a proliferous rose, confirms Van Tieghem's opinion as to the nature of the rose tube. Bonnier and du Sablon (1905) present the same opinion as Van Tieghem. Hillmann (1910) studied the vascular structure of the tube of rosaceous flowers (in cleared specimens) and concludes that the tube consists of congenitally concrescent leaf structures except in *Rosa*, where it is wholly axial. Hillmann's paper does not make clear (to the author at least) why he considers the tube of *Rosa* entirely axial, or just what leaf structures he considers are included in the tube of other rosaceous flowers. Bonne (1928) made a study of the vascular anatomical structure of a large number of rosaceous flowers. She concludes that the vascular evidence shows the tube to be appendicular, formed of the united basal portions of the sepals, petals, and stamens, in all rosaceous flowers except *Rosa* (and *Prinsepia*, *Nuttallia*, and *Chamaebatia*). In *Rosa* the vascular structure shows, she says, that the lower part of the tube is axial, the upper part is appendicular and similar to the tube of other rosaceous flowers.

As far as the present writer has been able to ascertain, the receptacular nature of the tube is supported only by the ontogenetic evidence and by Rydberg's statements. The fact that the fusion of the basal parts of the sepals, petals, and stamens does not appear in ontogeny is not proof, however, that such a fusion has not taken place, for this fusion is considered to have taken place in phylogenetic development. Rydberg's statement that the bundles of the sepals, petals, and stamens are united in the tube does not hold in all cases. Furthermore, comparative anatomical study shows that the fusion of these bundles, in the flowers which show it, has come about in the

evolutionary development of those flowers. The abscission of the petals at their points of apparent insertion on the margin of the tube must be considered a specialization in floral development.

Except for Velenovský and Domin, those (of the writers mentioned above) who call the tube calyx offer no evidence to support their statements. The belief of Velenovský and Domin in the calyx nature of the tube is based largely on study of teratological specimens. Although the value of such evidence is not denied, it seems, to the present author, that an attempt to explain normal structures largely on the basis of abnormalities is fraught with great possibility of error. The present author has not examined material similar to that described by Velenovský and Domin, but from the descriptions given by them it seems to the present author that these abnormalities, if studied anatomically and in comparison with normal forms, could readily be explained in harmony with the interpretation of the tube presented in this paper. Furthermore, the belief that the tube is calyx is incompatible with its observed vascular structure.

Those who consider the tube to be a structure composed of the fused basal portions of the floral organs outside of the carpels, except in the case of *Rosa*, where the upper part of the tube is held to be of this nature and the lower part axial, have offered the vascular structure of the tube as evidence that this is the case.

The conclusions of the present writer as to the nature of the tube in rosaceous flowers with free gynoecium are in agreement with the opinions of Van Tieghem, Bonnier and du Sablon, Bonnier, and Bonne, except with respect to certain minor details. The writer does not, however, entirely agree with the vascular anatomical descriptions given by Van Tieghem and Bonnier.

In the present paper use of the terms calyx-tube, receptacular-tube, and hypanthium has been intentionally avoided. These terms are used in papers, taxonomic works, textbooks, etc., to refer to the tube-like part of rosaceous flowers (and certain others). The author is of the opinion that the use of these terms should be discontinued. The first two, because they imply that the tube is calyx or receptacle, and the study of its vascular structure shows that this is not the case. The third, because the tube is not a structure below the flower. It is very much a part of the flower. The term floral tube is offered as a substitute, for the tube is obviously a floral structure.

SUMMARY

A comparative study was made of the vascular structure of the flowers of *Rosa* and of certain other rosaceous genera with superior gynoecium to ascertain, if possible, the morphology of the tube of these flowers. From the information obtained in this study the following conclusions are reached:

The tube of these flowers with the exception of *Rosa* is appendicular in nature and is formed of the fused basal portions of the floral organs outside of the carpels. It consists, thus, of the lower portions of the sepals, petals,

and stamens (and sometimes bracts) which have become united in phylogenetic development into a tube of varying length.

The apparently free sepals, petals, and stamens (and sometimes bracts) which appear to arise on the margin of the tube are merely the non-united distal portions of these organs.

The position of the carpels on the receptacle at a level lower than that of the other floral organs (in many cases) is only apparent and has been brought about by the formation of the tube.

Subsequent to the external fusion of the basal parts of the sepals, petals, and stamens (and bracts), there has taken place in some cases an internal fusion of the vascular traces to these organs. This fusion is present in various stages in the flowers of the Rosaceae. A series of forms is presented showing stages in degree of completeness of this vascular fusion.

The lower part of the tube of the rose flower is axial, the upper part appendicular. In the phylogenetic development of the rose flower invagination of the terminal portion of the floral axis has taken place. This process has brought the morphological apex of the floral axis to the bottom of the hollow in the rose flower, and has caused the inversion of the stelar bundles of the invaginated portion.

The upper part of the tube of the rose flower is appendicular in nature and is homologous with the tube of the other rosaceous flowers discussed. As in those flowers, it consists of the united basal portions of the sepals, petals, and stamens. The distal portions of these organs are non-united and appear as free organs.

The position of the carpels of the rose on the receptacle at a lower level than that of the other floral organs is only apparent and has been brought about by the invagination of the carpel-bearing part of the floral axis and by the formation of the upper part of the tube by fusion of the basal parts of the sepals, petals, and stamens.

The line separating the axial and appendicular portions of the rose tube can be determined only approximately. It lies at a level somewhat above the highest point of bending back of the stelar bundles in the wall of the tube. This level varies in different species of roses. In the species described it falls at about the middle of the tube-like part of the flower.

The terms calyx-tube, receptacular-tube, and hypanthium, which are used to designate the tube-like part of rosaceous flowers, are inaccurate and misleading. The writer suggests that the term floral tube be substituted for them.

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HISTORY OF LEAF DEVELOPMENT IN *BRYOPHYLLUM CALYCINUM*

JOHN A. YARBROUGH

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Numerous physiological studies of so-called "regeneration" in the *Bryophyllum* leaf have been undertaken in the past, particularly within the last fifteen years, but the results are difficult of correlation. Recently this leaf has been the subject of several morphological studies. Interest in these papers has centered upon the latent meristem of the leaf notches and its relation to the vegetative propagation of the plant. Howe (1931) emphasized the normal occurrence of meristem in the leaf notches and its definite organization into "buds" upon adult leaves. She stated that the roots of the young plantlets take their origin "somewhere near the vein," and are formed some time after the stem primordium. Mehrlich (1931) in a recent paper included some observations on the morphology and general structure of the leaf. He found that "marginal, sub-epidermal meristems" existed in young leaves one-eighth of an inch long and concluded that proliferation of the leaf does not involve the changing of differentiated cells into the embryonic condition.

The writer (1932a) in a preliminary report on the *Bryophyllum* leaf pointed out that latent meristems are present in leaves but 2 mm. in length and give rise in adult leaves to "foliar embryos" comprising primordia of both root and shoot. Since residual, localized meristem thus occurs normally in the notches of the leaves, it was argued that the term "regeneration" should not be applied to the development of such embryos. Naylor (1932) reported the normal occurrence of definitely organized primordia in the *Bryophyllum* leaf and applied the term "embryo" to such units. His conclusions agree with those of Howe as to the origin of this foliar meristem; but Naylor also described root primordia and a "foot" region for embryos on attached, normal leaves. Notches were reported by Naylor as first appearing when leaves were 5-6 mm. long. The formation of such notches was correlated with the establishment of the meristem in the leaf. More recently the writer (1932b) published a developmental and anatomical study of the foliar embryos in *Bryophyllum* which included, however, only a general survey of leaf development. It was pointed out that foliar embryos are laid down very early in ontogeny and are to be correlated in time with the basipetal notch development.

The appearance of these five articles within the past two years indicates a considerable interest in the morphology of the *Bryophyllum* leaf. It is

unusual that in the large number of papers concerning this leaf, both physiological and morphological, appearing within the last twenty years apparently only Ossenbeck (1927) mentioned the work of Berge (1877) which covered in a rather thorough manner the history of the foliar meristem recently described by Howe, Naylor, Mehrlich, and Yarbrough. The obscurity of this publication probably accounts for its having been overlooked by modern workers. Berge's dissertation, published in 1877, described the origin of "buds" upon the leaf and the development of stem and leaf primordia therefrom. He made it clear that these buds are exogenous in origin and are definitely associated with the leaf notches. He held erroneously that further development of these buds occurs only on detached leaves placed in a moist environment, roots appearing only on such detached leaves and arising from the cambium of the vascular bundle contiguous to the foliar meristem. Berge also described the hydathodes which occur in the lobes of the leaf and which consist of an epithem associated with a special group of stomata upon the lower surface of the leaf. His experiments showed that exudation of liquids may take place to a considerable degree through these hydathodes. Berge dealt also with the development of the leaves, emphasizing the basipetal appearance of lobes and major vascular strands. He discussed the development of the stem and the course of vascular bundles through it, matters which are not related to the present discussion. This early worker has presented in many respects a very clear picture of the development of the *Bryophyllum* leaf as well as the relations of the leaf to the plantlets arising from it. It is unfortunate that this worthy contribution has been overlooked by so many recent students of the problem, especially since several of the later conclusions are essentially repetitions of those of Berge.

The present study is an attempt to follow critically the development of the *Bryophyllum* leaf with special reference to the history of germinal layers or tissues. It deals with this problem by using the methods of embryology in order that this rather unusual leaf may be properly interpreted as a functional organ of the plant body. Much credit must be given to the work of Berge, but the writer feels that some criticisms of this early work are necessary and additional conclusions are justified. The writer also analyzes the various published interpretations of the phenomenon of plantlet development from the *Bryophyllum* leaf in the hope that there may result a clearer understanding of the morphological principles involved.

MATERIALS AND METHODS

Materials used in this work were secured from plants growing in the plant houses at the University of Iowa. The bulk of the material surveyed was *Bryophyllum calycinum* Salisb. (*B. pinnatum* (Lam.) S. Kurz; *Kalanchoe pinnata* Pers.); however, leaves of *Bryophyllum crenatum* Bak. (*Kalanchoe crenata* R. Hamet) were also used for comparative study. The *B. calycinum* plants were secured from the Department of Botany of the Uni-

versity of Chicago and the *B. crenatum* plants from the Missouri Botanical Garden, St. Louis.

Portions of leaves of various sizes and entire stem tips were killed in Flemming's weaker and in formalin-acetic-alcohol and prepared by the usual paraffin method. Delafield's haematoxylin was the most useful stain employed. A dissecting binocular microscope was used in surveying the gross morphology and configuration of leaf primordia. Leaves were cleared for venation study by the use of chloral hydrate. Sections of young leaves were cut in three planes, cross, longitudinal (at right angles to the leaf surface), and paradermal (parallel with the flattened leaf surface). Sections cut in this last plane have been designated "horizontal" or "tangential" by various authors. If a term is to be used which correctly describes such a section of the youngest leaf primordia often closely appressed to the cylindrical stem tip, "tangential" would seem the logical one. If, however, we employ a term which more obviously describes this section of the adult leaf, then "paradermal" would seem appropriate, since this word is merely a contraction of the descriptive phrase, "parallel to the surface of the leaf," so often used in explaining the terms "horizontal" and "tangential."

RESULTS

The shoot system and the mature leaf

The stem of *Bryophyllum* bears opposite, decussate leaves. The first 15-20 pairs of such leaves borne by a plant are normally simple. Older leaves are compound, possessing 3 or 5 leaflets (fig. 5, pl. 1), which is considered the normal leaf organization according to Engler and Prantl (1930). Leaflets and simple leaves are elliptical with a crenate margin. Variations from the normal configuration in the earliest formed leaves of a plant and in the hypsophylls have been described by the writer (1932b). Small, scale-like leaves which appear on sucker shoots arising from roots have few crenations (fig. 9, pl. 2), yet conform to the general shape and organization of the normal foliage leaf. There is considerable variation in leaf size. Leaflets may range from 5 to 13 cm. in length, while simple leaves upon vigorous plants may become even larger. Both simple and compound leaves are petiolate, compound leaves usually having the longer petioles. There is more or less extension of the blade upon the petiole at their junction to form short wings. The petioles widen out into the leaf bases, and the pair at a given node finally completely encircle the stem to form a collar (fig. 3, pl. 1).

The mature leaf, seen in section, presents a relatively simple structure (fig. 7, pl. 1). The large, thin-walled cells which compose the mesophyll are not differentiated into spongy and palisade layers. Vascular bundles with normal xylem and phloem orientation ramify this mesophyll. Contrary to the condition in many thin-leaved dicotyledons, these bundles do not lie in exactly

the same plane, a condition which might be expected in a leaf with massive, undifferentiated mesophyll. The two epidermal layers both bear stomata. However, they are more numerous on the lower surface, averaging about 120 per sq. mm. as compared to 72 per sq. mm. on the upper surface. These figures include some immature stomata found even upon leaflets 9 cm. long. Weiss (1865) reported 50 and 67 stomata per sq. mm. for upper and lower surfaces, respectively, for the leaf of *Sedum latifolium*. It will be noted that these succulents have relatively fewer stomata than many thin-leaved dicotyledons with stomata only on the under surface.

The idioblasts which are present in the mesophyll (fig. 7, pl. 1) and petiole as well as in the stem and root contain anthocyanin pigments, as shown by tests with acid and alkali. These same cells also tested positively for tannins by the methods described by Zimmermann (1893). No positive evidence of the presence of mucilage in them could be found by the simpler methods available, though its presence has been reported by Lund and Bush (1930). However, it seems probable that anthocyanin pigments and tannins are associated with mucilage or closely related carbohydrate material in these idioblasts.

The venation of the leaf seen plainly in cleared leaves is of the pinnate type. A strong mid-vein and two or more small laterals enter the base of

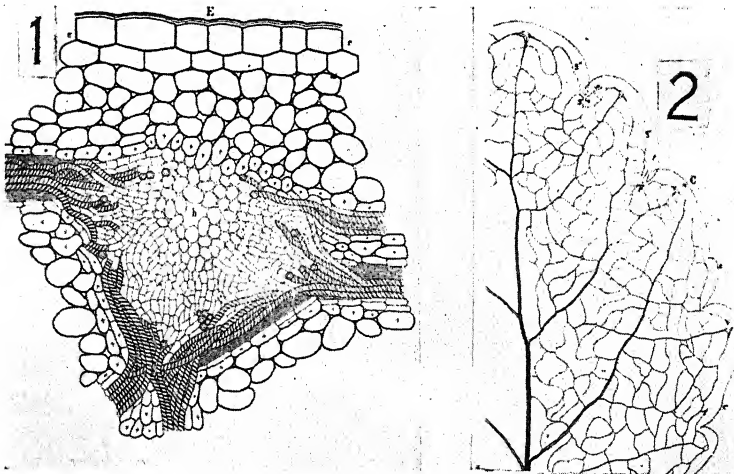


Fig. 1, 2. Fig. 1, paradermal section of hydathode of a leaf. After Berge. $\times 85$. Fig. 2, right half of leaf showing relation of venation to hydathodes. After Berge. \times approx. $\frac{1}{2}$.

the blade (fig. 8, pl. 2). The laterals branch little and are diverted to the lower lobes, while the central mid-vein gives off the bulk of the branching veins of the leaf proper. The node is a trilacunar one, as noted previously (Yarbrough, 1932b). There are no vein endings in the margin of the normal

foliage leaf, but the peripheral branches all terminate more or less directly in a plexus of vascular elements located in the lobe of the blade (fig. 1). These areas were termed "apex patches" by the writer (1932b) before their true hydathode character was determined by him. Berge's article, discovered shortly afterward, had previously described their function as hydathodes. The epithem of these structures is composed of small cells containing some chlorophyll and prominent nuclei and seeming almost meristematic in appearance, though there was never any evidence that proliferation takes place from these regions. Such power seems to be resident only in the mass of meristem found in the sinus of each crenation.

Early development of primordia

The leaf primordia appear in pairs as dome-shaped, papillary outgrowths of the bluntly conical growing point. Since the formation of each primordium involves a considerable number of cells, the growing point is almost completely used up as each pair develops (fig. 1, pl. 1), and the plastochron in this case is thus relatively long as compared with that in some thin-leaved dicotyledons—e.g., *Helianthus*, *Coleus*. A considerable time interval must elapse between the appearance of successive pairs of primordia in *Bryophyllum*, during which the apical meristem is regenerated. Comparative measurements of primordia up to 7 mm. long indicate a ratio of about 20 or 30 to 1 in length of successively formed pairs. It must be recognized that this ratio is a statement of the plastochron in terms relative to size only, whereas the interval is actually one of time. An evaluation of the plastochron in time units would perhaps be desirable, though difficult and often inconstant due to environmental variation during growth as well as to varying ages of shoots. As Schuepp (1929) has pointed out, the plastochron is really dependent upon two factors: (1) the rate of mitosis in the growing point and (2) the size relationship between the youngest foliar primordia and the remaining portion of the stem tip. It seems that in *Bryophyllum* the second is the determining factor.

The first of the germinal layers to be recognized in the leaf primordium is the dermatogen. There is little difference, however, in the size of dermatogen cells as compared with the size of the remaining cells, each having a diameter of about $6.5\ \mu$. The pro-mesophyll cells have more facets and do not show the regular arrangement exhibited by the dermatogen. Mitosis is, of course, occurring throughout the young primordium increasing its length, width, and superficial area. For a considerable time during this expansion the dermatogen cells retain the size stated above. On the contrary, enlargement and vacuolization of cells in the pro-mesophyll bring them rather early (primordia $50\ \mu$ in length) to a diameter of $8-10\ \mu$.

The first indication of desmogen differentiation comes when the primordium is between 60 and $70\ \mu$ long as typical elongate cells appear slightly adaxially to the center of the primordium. These cells represent the median

leaf trace and develop acropetally into the enlarging primordium and basipetally into the tissue below establishing connection with the stele (fig. 1, pl. 1). The two lateral leaf traces do not make their appearance until about the 375μ stage, arising left and right of the median trace and joining it near the meristematic tip of the primordium (fig. 6, pl. 1). Related to the development of this initial desmogen strand is an obvious difference in the promesophyll on either side of it. Abaxially the cells enlarge faster than those which are adaxially related to the median trace, a condition apparent as early as the 160μ stage, when the abaxial cells may be twice the size of those in the other half of the primordium (fig. 4, pl. 1). It seems possible that the smaller cells of the adaxial half with their denser cytoplasm may explain Mehrlich's observation of a staining difference between the two halves of the young leaf. At this stage there are 8-10 layers of cells in the pro-mesophyll, and there may be 1 or 2 fewer layers adaxial to the first desmogen strand than abaxial to it. Though it is virtually impossible to recognize layers of cells in the adult leaf, there does seem to be a corresponding displacement of many of the veins above the midplane of the mesophyll. It is evident that the earlier expansion of cells in the abaxial half of the primordium results in the cupping or folding of the wings as they develop. Thus the two (opposite) young leaves are closely appressed with their wings interlocking (fig. 4, pl. 1). Much protection of the growing point is undoubtedly afforded by this situation, as was observed by Berge (1877). As the leaves mature, the cells of the adaxial half expand, and thus the wings of the lamina flatten out into their normal adult condition.

Berge observed that there was no terminal or apical growing tip of the leaf. Though this is true if only the later stages of leaf development are considered—i.e., 1 mm. and above—the early primordium does bear a conspicuous apical meristem. The disappearance of the apical growing region seems to be correlated with the development of wings and hydathodes, which will be discussed later. Localization of meristem along the lateral edges of the primordium is evident as early as the 350μ stage or about the time the lateral leaf traces appear, and represents the beginning of wing formation, which proceeds rapidly from the time the primordium is 500μ long.

Later development of primordia

Beyond the 500μ stage further development into the adult condition will be followed largely through changes in external form and configuration, considering only normal young leaves of the tenth node and above. Internal changes incident to development into the adult form will be discussed in later sections. Shortly after the initiation of wing formation in the primordium of a simple leaf, crenations appear in these wings in a basipetal fashion as described by Berge (1877) and Yarbrough (1932b). If the primordium is to develop into a 3- or 5-pinnate leaf, however, the lobes which form the lateral leaflets appear previous to any crenations in the terminal leaflet (fig.

14, pl. 2). The appearance of the latter, however, quickly follows that of the former (fig. 11, pl. 2). Berge stated that the lateral leaflets appeared after the basal crenations of the terminal leaflet, but my work does not bear out this conclusion. On the contrary, it seems that the leaflets appear in acropetal order and the crenations of each leaflet in basipetal order. Trecul (1853) has shown that this is the case in other forms—i.e., the members of succeeding series do not always appear in the same order.

There is disagreement in the literature as to the time of appearance of crenations in the leaf primordium. Naylor stated that they appear first upon leaves between 5 and 6 mm. in length. Berge's account leads one to think he would place the time of appearance of crenations at about the 7 mm. stage. My work indicates the presence of crenations much earlier, even upon primordia only 2 mm. in length. If this is true, then the origin of the foliar meristem may be found much earlier than the work of Naylor and Berge indicated, for the appearance of this structure is directly associated with the development of crenations. The continuity of meristematic tissue from the stem growing point to the foliar meristem is thus clearer if crenations appear as early as indicated above. The development of secondary lobes has been rather thoroughly discussed by Berge. They occur more often upon the terminal primary lobes, rarely, if at all, upon basal lobes of the leaflet or leaf, and develop in basipetal sequence as do the primary lobes.

As wing and crenation development proceed the petiole arises out of the base of the primordium with the wings of the lamina often slightly decurrent upon its upper portion. In the case of compound leaves the rachis also is appearing at the time of petiole development, and the former structure lengthens into the adult form.

The variation in leaf form in *Bryophyllum* from simple to pinnately compound has been discussed by Hofmeister (1870), Berge (1877), and Engler and Prantl (1930). Simple leaves do not normally occur above the twentieth node of a plant; yet 3-5-pinnate forms occur above this point in no apparent order of succession. If the terminal growing point is removed from a shoot which is regularly producing compound forms, the two topmost axillary buds will develop almost simultaneously. In several such experiments the writer has found that the first pair of leaves formed was always simple, but the second and immediately succeeding pairs were of the compound form. Thus, while the development of the axillary bud does not repeat the exact succession of leaf form of the plantlet, it is worth while to note that the first pair of leaves of the axillary shoot does show the simple form.

The epidermal system

The dermatogen cells keep pace by repeated mitoses with the expansion of the primordia and retain their original size until about the 500 μ stage. Their enlargement, begun at about this time near the tip of the primordium, seems to be correlated with the disappearance of the apical meristem there (fig. 2,

pl. 1). Mitoses continue, however, in dermatogen cells below the primordium tip until relatively late in leaf development. Adult epidermal cells are irregular in outline, seen from the surface, with an average diameter of $72\ \mu$ and an average thickness of $30\ \mu$, though there are wide fluctuations from these averages. Comparing these measurements with those of the dermatogen cells, we see that they have increased in volume more than 440 times. Cuticle on the epidermis is about $2\ \mu$ thick. Prominent on the margin of young leaves are the inflated epidermal cells (fig. 4, pl. 1), which first appear on the tip of the terminal lobes and then successively upon the lower lobes. As Berge pointed out, these are temporary structures on the stem and other parts but commonly persist on the leaf, where they invariably occur along the margin. Nuclei are evident in them until the leaves are several centimeters in length. In only one case were trichomes observed on leaf primordia. They occurred on the adaxial surface of leaves about 5 mm. long and were multicellular with a gland-like tip (fig. 10, pl. 2). No trichomes seem to have been reported previously as occurring on either immature or adult leaves of *Bryophyllum*. Their presence on young leaves of other forms which in the adult condition are glabrous, or nearly so, is not unusual and in this case may have been due to injury or infection.

As mentioned above, stomata occur on both epidermal surfaces. These are of the normal type with conspicuous subsidiary cells and sub-stomatal air chambers (fig. 17, pl. 2). In surface view the adult stoma shows several subsidiary cells (fig. 15, pl. 2). Mature stomata do not usually appear previous to the 7-8 mm. stage except in connection with the development of the hydathode, to be discussed later. The stomatal system is in process of development before the 7 mm. stage, and developing stomata may occur much later, even on leaves 9 cm. long. The development of stomata occurs in much the same way as that described by Strasburger (1866) for *Sedum spurium*. Berge (1877) referred to stomata on *Bryophyllum* as being formed by spiral preparatory divisions but did not describe the details. The guard cell initial arises by a normal division of an epidermal cell (fig. 16, pl. 2). The first division of this initial is unequal, cutting off a cell laterally by a curved wall. The next division similarly forms a second cell with a curved wall approximately perpendicular to the plane of the first division. Succeeding divisions of the initial occur in the same manner, thus laying down a spiral series of four or five cells which in turn enlarge and vacuolate (fig. 13, pl. 2). In this series of mitoses it is always the inner cell which remains capable of division and which is constantly decreased in size. The final division of the "Specialmutterzelle" produces the two guard cells seen in the adult condition (fig. 15, pl. 2). The guard cell initial thus behaves very much as an apical cell with three cutting faces. Such a type of stomatal development seems to be characteristic of the Crassulaceae, according to Strasburger, who also recorded his observation of several cases in *Sedum spurium* where one of the subsidiary cells of the spiral series retained its power of division and became a guard

cell initial which began its own series of spiral subsidiary cells. No such case was found in my work on *Bryophyllum*.

Development of the vascular system

The description of the median and lateral leaf traces showed the condition of vascular differentiation up to the 700–800 μ stage. Further differentiation is intimately related to the appearance of the hydathodes and to the development of the leaf margins with their crenations. When the apical growing tip disappears at about the 700 μ stage, a group of meristematic cells is set apart in that portion of the primordium (fig. 2, pl. 1). These cells constitute the pro-hydathode, which is in direct connection with the median leaf trace, representing in fact the apical termination of that desmogen strand. At this stage lateral growth of the wings of the lamina is proceeding rapidly, and coincident with the organization of the pro-hydathode the first crenation appears in the apical half of the leaf primordium. The first tracheids to be differentiated appear in the median trace (fig. 2, pl. 1) and establish a direct connection between the pro-hydathode and the stele below.

As mentioned above, the two lateral traces even in this early stage are united with the median trace in the upper half of the primordium, this compound strand later forming the mid-vein of the leaf. Two desmogen strands appear, left and right of this mid-vein, and run in a broad curve from the pro-hydathode downward joining the mid-vein near its basal end. As other crenations are developed, a pro-hydathode is formed in the apex of each, and it is from these centers that vascular differentiation proceeds in the basipetally appearing crenations of the young leaf. The events of development in each lateral lobe repeat those of the apical lobe, at least in a general way. The two lateral, peripherally curving veins, described above, are not always apparent in their basal connections (fig. 2); yet the hydathode does usually receive three veins, one fairly median to its lobe and two laterals. Berge pointed out that the marginal vascular strand which connects adjacent hydathodes and runs close by the notch meristem develops relatively later than those belonging primarily to the apex of the crenation itself. This means that the notch meristem or foliar embryo anlage becomes connected with the general vascular system relatively late. In summarizing the manner of vascular differentiation the most significant facts observed are: (1) the early differentiation of the mid-vein which ends peripherally in the terminal hydathode; (2) the basipetal appearance of hydathodes in lateral crenations; and (3) the general basipetal differentiation of the minor veins about these as centers.

The adult vascular bundles of the leaf consist of reticulate tracheids and elongate, thin-walled phloem cells. These latter cells average about 70 μ in length and 7.5 μ in diameter. The tracheids, on the other hand, may be as much as 40 times as long as they are wide. Normal orientation of parts with adaxial xylem and abaxial phloem occurs throughout the leaf except in the

region of the sinus, where, as Naylor (1932) has shown, the xylem is displaced outward toward the margin of the leaf. The foliar embryo is located between this vein and the leaf margin, and Naylor concluded that the decided expansion of cells adaxial to this meristem, compared to that of the meristem cells themselves, accounts for the twisting of the xylem from its normal position.

Berge did not outline the early development of the hydathodes. The first of them may appear when the leaf primordium is $700\ \mu$ to $1000\ \mu$ long, represented at that time by the pro-hydathode described above. Approaching vascular differentiation intrudes on all save the abaxial side of this pro-hydathode and soon separates it from surrounding mesophyll cells. An interesting fact which Berge did not mention is that stomata are formed on the abaxial surface of the young leaf opposite the earliest formed hydathodes before stomata appear elsewhere on the leaf surface. A group of 4-5 mature stomata as described by Berge for the adult leaf may appear opposite the young hydathode when the leaf is only $2\frac{1}{2}$ mm. long, whereas adult stomata do not normally appear on the general leaf surface previous to the 7 or 8 mm. stage. The hydathodes are active in very young leaves, and this fact is undoubtedly correlated with the early appearance of stomata opposite the main body of the hydathode. The pro-hydathode, or original mass of meristem, increases considerably in volume. Mitoses were observed in the apical hydathode of a 3 mm. leaf with highly vacuolated cells surrounding the meristematic mass in which cell division is probably continued even longer. The epithem cells of the hydathode remain approximately isodiametric, contain some chlorophyll, retain prominent nuclei, and do not increase much in size. Mature cells may range in diameter from 10 to $13\ \mu$, while some are only 6 or $7\ \mu$ in diameter or the size of meristematic cells of the leaf. The organization of the adult hydathode is clearly shown in one of Berge's drawings (fig. 1). Structures of analogous function have been described in two other forms, *Lafoensia nummulariifolia* by Ross and Suessenguth (1925) and *Campanula rotundifolia* by Rea (1921). Solereder (1908) refers to the observations of several early workers upon the "water pores" in *Bryophyllum* and other genera of the Crassulaceae. Variations of this general type of hydathode structure are to be found in a number of species, according to Haberlandt (1914).

The vascular organization of the petiole deserves a brief description. As mentioned above, the two lateral traces unite with the median trace just above the node to form the main crescent-shaped strand of the mature petiole (fig. 12, pl. 2) which emerges into the lamina as the mid-vein. The situation is somewhat complicated, however, by the branching of the lateral traces before their union with the median trace or even by the branching of the median trace itself. These small branches maintain their independent course through the petiole, may in turn branch, and end in the lower portion of the lamina (fig. 8, pl. 2). Their number, exact position, and orientation do not seem at all constant (fig. 12, pl. 2).

The mesophyll

Because of its homogeneous character the mesophyll of the adult *Bryophyllum* leaf presents fewer problems in the study of its developmental history than that of a typical mesophytic leaf. Large, thin-walled, parenchymatous cells compose the entire mesophyll, which is not specialized into layers comparable to palisade and spongy parenchyma. Mitoses occur uniformly throughout the pro-mesophyll and are continued longest in the vascular bundle regions, as Mounts (1932) has shown for *Vitis* and *Catalpa*. The idioblasts appear early in leaf ontogeny, being frequently more numerous just beneath the epidermal layers. The early differentiation of abaxial and adaxial halves of the leaf has been discussed. With the flattening of the wings of the lamina this difference in cell size in the two halves of the leaf largely disappears. The thickness of the adult leaves varies greatly, but in general those produced in the winter months are much thicker than those formed in the warmer season. Leaves may vary from 560 to 2500 μ in thickness and in both extremes have approximately the same number of cell layers, the cells in the thick "winter" leaves being greatly increased in size. Adult mesophyll cells may thus vary in diameter from 50 to 300 μ .

The differentiation of the minor veins of the blade organizes the mesophyll into areas of tissue commonly known as vein islets. These vary from triangular to quadrilateral in shape and average about .12 sq. mm. in area. Smaller islets may have an area of .028 sq. mm. and the larger an area of .18 sq. mm. The intervacular interval or approximate diameter of such vein islets is found to vary from 150 μ to 600 μ and to average about 300 μ . The average maximum distance of mesophyll cells from a vein then is about 150 μ , and most cells are nearer to conduction channels. There are no small intruding veins or twig ends in the islet tissue such as are found in many thinner leaves.

In spite of its unusual thickness the exposed internal wall area in the adult *Bryophyllum* leaf has been found to be considerably smaller than the corresponding values for several other leaves. The writer is indebted to Mr. F. M. Turrell for unpublished data upon this point.¹ Turrell (1932) has calculated the ratio of internally exposed cellular surface to external epidermal surface for several leaves, using methods described in detail in his thesis. His figures for *Bryophyllum calycinum* show a ratio of 7.4/1, the lowest found in any leaf yet measured by him. This condition is suggested if sections of the leaf (fig. 7, pl. 1) are compared to similar sections of such a leaf as *Vitis* or *Rhus*. It would seem that the uniformly isodiametric cells in the mesophyll of the *Bryophyllum* leaf account for its small internal exposed surface.

The intercellular space system of the leaf consists of connected series of chambers bounded by the internal cellular surface described above. The

¹ Since the preparation of this manuscript, Turrell has published portions of his data in *Science* and in the *University of Iowa Studies*.

spaces are pyramidal or roughly triangular in cross-section, occurring at the place where the walls of three contiguous rounded cells meet (fig. 3). The bounding walls of the spaces are often invaginated into the cells rather than

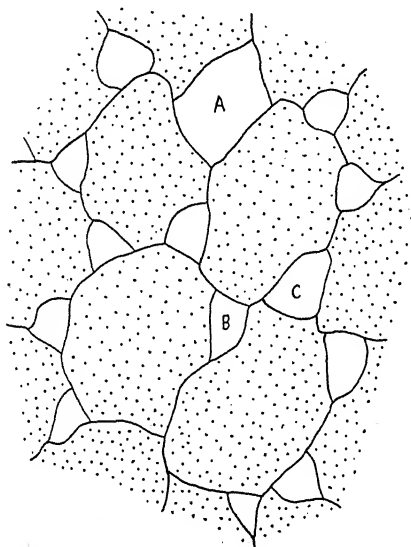


Fig. 3. Paradermal section of mature foliage leaf showing intercellular spaces. Space *A*, bounded by four cells, is the result of fusion of two spaces like those shown at *B* and *C*. Fig. 7, Pl. 1, shows the vertical extension of some of the spaces. Further separation of cells in the *Bryophyllum* leaf might result in the condition observed in spongy parenchyma of typical thin-leaved dicotyledons. $\times 182$.

being convex, a condition commonly observed in spongy mesophyll of leaves and in many parenchymatous tissues. One of the factors producing the spaces in the *Bryophyllum* leaf is probably the unequal expansion of epidermis and interior tissues, as described by Mounts (1932) for other forms. She has shown that for *Vitis vulpina* the epidermal cells increase about 20 times in surface area, while the underlying palisade cells increase only about 8 times in cross section area. It is evident that this lateral strain operating through both upper and lower epidermal layers would tend to separate mesophyll cells, as Mounts asserts. The cells of the epidermis in the *Bryophyllum* leaf are relatively late in expanding, mitoses continuing there after having ceased in the mesophyll. Moreover, this expansion of epidermal cells is largely in the paradermal direction, and the tension thus created would appear to be an important factor in the formation of intercellular spaces. The invagination or concavity of the bounding walls, however, is difficult of explanation.

The most important specializations associated with the mesophyll are the foliar embryos, one of which is organized in the sinus of each crenation. Their development has been rather thoroughly followed, first by Berge (1877) and then by several later workers as mentioned above. There is general

agreement that these foliar meristems are of normal occurrence in the leaves of this plant and represent only a rather unusual method of plant propagation. It is of interest to note that in two species of *Kalanchoe*, a sister genus of *Bryophyllum*, the foliar embryos are not present, though the leaf organization is very similar to that in the latter.

DISCUSSION

This foliar meristem has been interpreted mainly in two ways: (1) as a "foliar bud" by Hofmeister, Berge, and Howe, and (2) as an "embryo" by Naylor or a "foliar embryo" by Yarbrough. Berge and Howe observed the formation of leaf primordia in the notches of normal attached leaves but contended that roots arose later following a stimulation to plantlet development. Howe stated that roots arose in the region of the vein which borders the meristem and according to Berge from the cambium of the above-mentioned vein. Naylor and Yarbrough agreed as to the general method of development of the leaf primordia but held that roots also are organized from the foliar meristem and thus traced the entire embryo to a common meristematic tissue. The term "embryo" is therefore employed by them rather than "bud," which was used by others. Naylor found two root primordia well organized in adult leaf notches and also an organ which he termed a "foot." The writer has repeatedly found root primordia in the notches of normal adult attached leaves, though he does not recognize the "foot" described by Naylor. Though root primordia may not always be found upon immature attached leaves, it is the writer's conclusion that at least the earliest roots of the plantlet arise from the foliar meristem, and this growing tissue may thus be aptly termed a foliar embryo. Secondary roots may arise from meristem associated with the vascular bundles, as described in detail by Berge (1877).

The problem of the origin of the foliar embryo seems clearly related to crenation development and hydathode appearance, events closely related in time and position. It has been previously shown that the first of these events is the appearance of the pro-hydathode and the vascular differentiation downward from it to the median leaf trace. Marginal expansion in the hydathode region follows closely, forming the crenation, and simultaneously the meristem in the sinus becomes evident. That this foliar embryo anlage arises from a group of cells and not from any single cell seems justified by my observations as well as those of Naylor (1932). A small mass of the meristematic tissue of the young leaf persists in the sinus, while surrounding tissue assumes the differentiated form. It may be recalled that connection of the foliar embryo anlage with the general vascular system is established relatively late. It is possible that this early vascular isolation may be a factor in the retention of residual meristem in the leaf notch. There are three questions involved in the causal aspect of the problem of foliar embryo development: (1) How is meristem retained in the notch during maturation of leaf tissue? (2) How is

this meristem (usually) inhibited at a certain stage of development on attached leaves? (3) How is the dormant foliar embryo stimulated to further and complete development? Physiological studies in the past have dealt only with the last and perhaps the most easily handled question. But the first two questions are equally pertinent ones when the developmental history of the leaf is understood. It has been repeatedly shown that plantlet development may occur on normal attached leaves of *Bryophyllum calycinum* under ordinary greenhouse conditions, and *B. crenatum* is even more prolific in this respect. Thus the development of the foliar plantlet would seem the natural expectation; its origin and inhibition at a certain stage are, on the contrary, the difficult problems.

In an attempt to interpret the morphology of this foliar organ, one is led to the possibility of considering the *Bryophyllum* leaf as a phylloclade. But its position, origin, vascular organization, limited growth, and basipetal differentiation constitute valid objections to such an interpretation. On the contrary, the ability of the leaf to retain meristem is not unique. The tendency is sometimes to disregard the many possibilities for variation resident in this lateral member of the stem. The existence of both root and shoot primordia, however, in the foliar embryos of the leaf notches suggests the rather extreme interpretation that in this case we may be dealing with structures which are the equivalents of vegetative, apogamous seeds. While dissociated from all floral organs, the foliar embryo in resting condition differs only in origin from the apogamous embryos of unquestioned seeds. The necessary growing points exist as well as available food from the parent leaf tissue. Protective coverings are not present, though there may be less need for them in the case of such a succulent. Even the development of the foliar embryo into a plantlet upon the parent leaf may be paralleled by some true seeds which germinate within the ovary in a viviparous manner. That the foliar embryos of *Bryophyllum* may be considered as apogamous seeds remotely comparable to such structures in *Hieracium* and *Taraxacum* was suggested by LeMaout and Decaisne, as noted in a previous paper (Yarbrough, 1932b). Further study leads the writer to call attention to the near approach in this form to what might be termed vegetative seeds.

In mode of origin, manner of differentiation, and general form the leaf of *Bryophyllum* corresponds to what might be expected as typical in succulent leaves. Throughout its normal span of activity it performs the typical functions of a leaf; yet an added function is present also, that of propagation. This is made possible through the foliar embryos borne in leaf notches and arising from meristem set apart very early in leaf ontogeny. Such function, though uncommon in the leaves of most plants, is none the less a normal and invariable one in *Bryophyllum*, in which all foliage leaves bear several potential plantlets. Moreover, this method of vegetative propagation is an extremely successful one, playing no small part in the rapid spread of the plant in its native and naturalized habitats, where it is often regarded as a pernicious weed.

SUMMARY

1. Leaf primordia arise in a decussate manner at the growing point with a long interval between the development of successive pairs.
2. The median trace, which differentiates at about the 70μ stage, is united near the tip of the primordium with the later appearing lateral traces. Vascular differentiation in the wings proceeds basipetally from the 700μ stage.
3. Crenations appear at about the 1–2 mm. stage and arise basipetally, hydathodes developing in the apices in the same order. Primordia of lateral leaflets arise acropetally.
4. Stomatal development is preceded by spiral preparatory divisions forming 4–6 subsidiary cells.
5. The internal exposed surface of the homogeneous mesophyll is small compared with that of other forms examined to date.
6. The development of the foliar embryos, which arise from meristem set apart very early in ontogeny, suggests the interpretation of the *Bryophyllum* leaf as an organ producing vegetative seeds.

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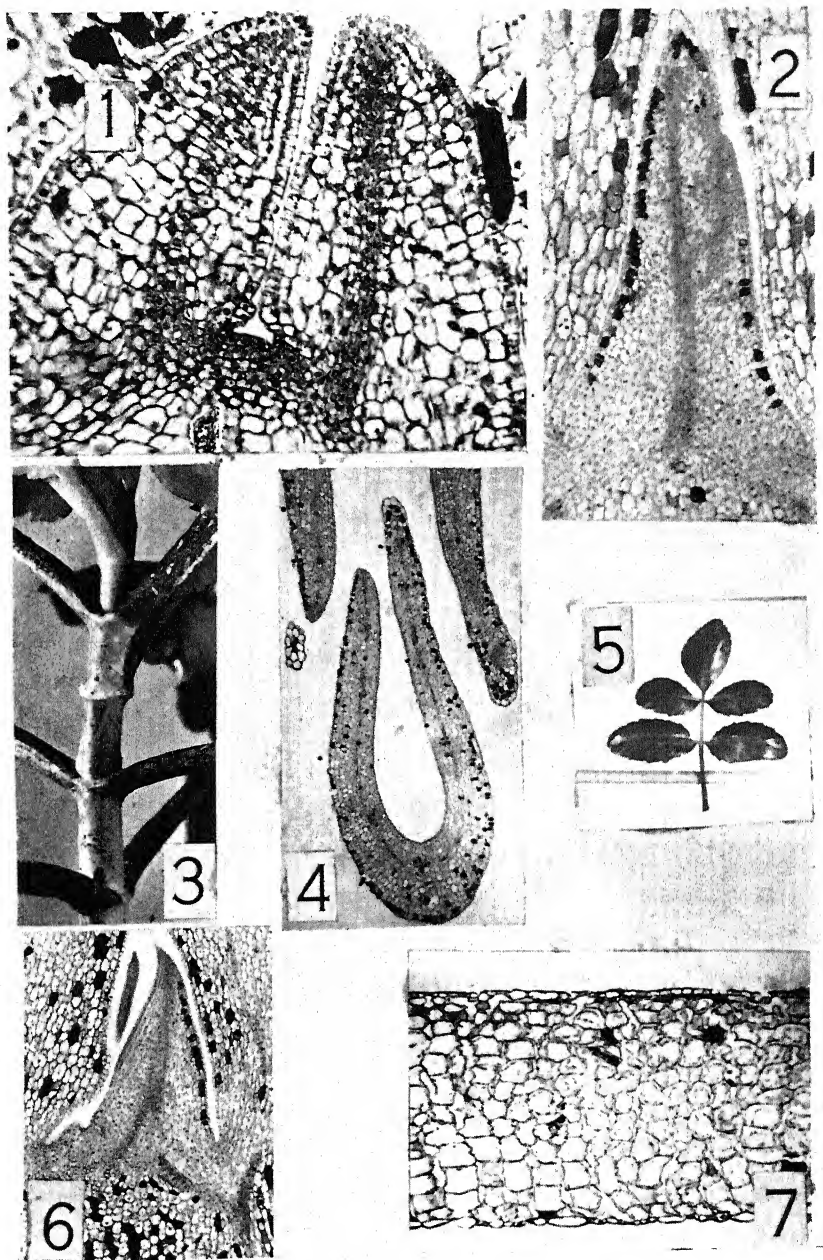
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EXPLANATION OF PLATE I

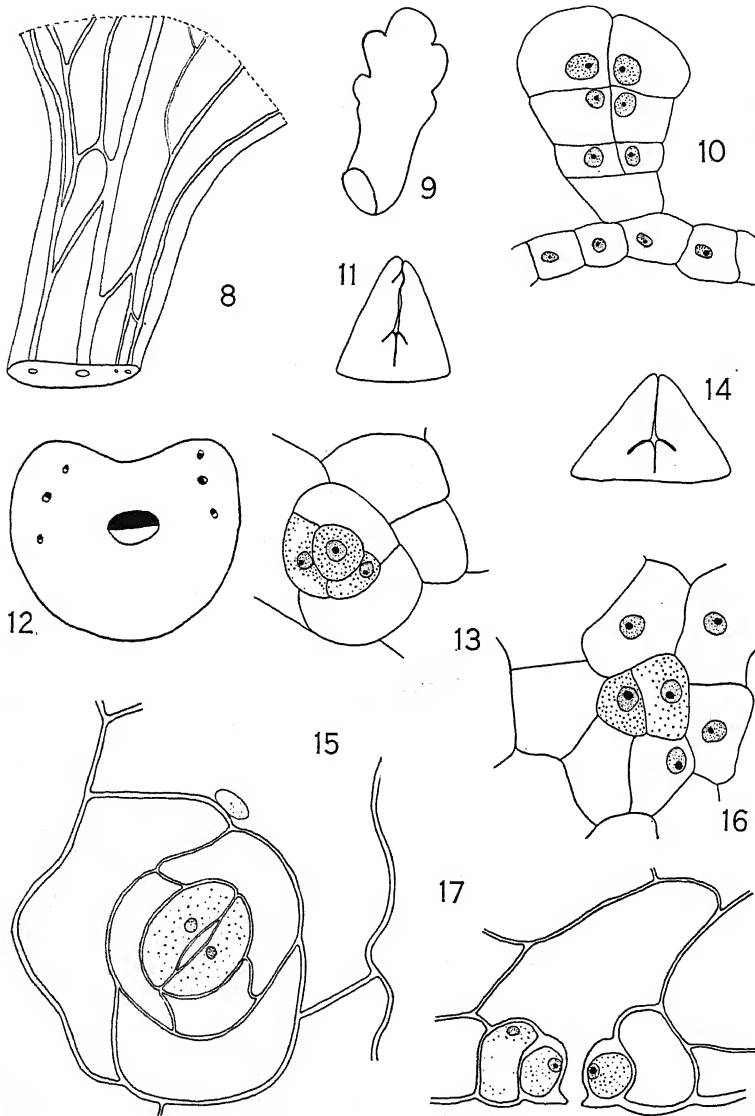
Fig. 1. Longitudinal section through growing point showing two young leaf primordia and "used up" stem apex. Note development of vascular strands. $\times 210$. Fig. 2. Paradermal section through a young leaf primordium showing median trace, initial hydathode formation, and appearance of first crenation. $\times 44$. Fig. 3. Stem showing leaf bases and petioles. $\times \frac{1}{2}$. Fig. 4. Cross section of young leaves showing inflated tip cells, hydathode, and position of vascular strand in relation to mesophyll. $\times 20$. Fig. 5. Compound leaf showing five leaflets. $\times 1/7$. Fig. 6. Paradermal section of young leaf primordium showing lateral traces united with median trace. $\times 29$. Fig. 7. Cross section of normal adult leaf. $\times 44$.

EXPLANATION OF PLATE 2

Fig. 8. Leaf base and petiole showing relation of vascular bundles. $\times 14$. Fig. 9. Small leaf found on sucker shoot arising from root. $\times 1\frac{1}{2}$. Fig. 10. Trichome found on adaxial surface of leaf primordium. $\times 660$. Fig. 11. Primordia of compound leaves showing leaflet primordia below and crenation development near tip. $\times 16$. Fig. 12. Diagram of cross section of petiole showing position and orientation of vascular strands. Xylem is shaded and phloem unshaded. $\times 16$. Fig. 13. Developing stomatal mechanism showing 4 cells in the spiral series and the central cell which will form the two guard cells. $\times 750$. Fig. 14. Primordia of compound leaves showing leaflet primordia arising previous to any crenation formation. $\times 30$. Fig. 15. Adult stoma in surface view. $\times 750$. Fig. 16. Developing stomatal mechanism showing first division of guard cell initial to form the first of spiral series. $\times 750$. Fig. 17. Cross section of adult stoma. $\times 660$.



YARBROUGH: BRYOPHYLLUM CALYGINUM



YARBROUGH: BRYOPHYLLUM CALYCINUM

FROST RING FORMATION IN SOME WINTER-INJURED DECIDUOUS TREES AND SHRUBS

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Winter injury, occurring either in late fall or early spring, has ever been a serious menace to vegetation, and the injurious effects frequently incurred by freezing are well known. The latter part of February, 1932, was characterized throughout Illinois by the prevalence of temperature conditions so exceptionally mild that growth activities were initiated in a number of kinds of woody perennials which would have maintained, otherwise, a more prolonged period of dormancy. The occurrence of a sudden freeze in early March, however, abruptly checked and injured this premature vegetative growth; and continuation of low temperatures into late March caused vegetative activity to remain in a state of dormancy for almost a month.

In the vicinity of Champaign and Urbana, winter injury was manifested in various trees and shrubs in that their subsequent growth was conspicuously altered upon the recurrence of temperatures favorable to growth. This injury ranged in extent from only slight to very severe and was exhibited particularly by elm, pussy willow, poplar, and privet, and to a lesser extent by maple and other plants.

In view of the general prevalence of winter injury the past spring, its occurrence in past years, and the probability of its recurrence in the future, it seems advisable to present herewith some studies and observations made in relation to winter injury in the Champaign and Urbana vicinity as an aid toward a fuller knowledge of its character. Accordingly, the general symptoms of winter injury are described, as well as some of the special symptoms exhibited by a few of the more severely and commonly injured trees and shrubs. Histological studies of frost ring formation in *Ligustrum* have been made, with a comparative histological treatment of *Ulmus*, *Populus*, and *Salix*.

CLIMATOLOGICAL DATA

The winter of 1931-1932 was the warmest yet recorded in the climatological history of Illinois, and any previous comparable winter antedates 1878. This was true for December, January, and February only, however, since March was more wintry than any of these preceding months. Soon after the first few days in March had elapsed, a sudden freeze occurred which was followed by the coldest week of the entire winter. It was this freeze, occurring March 5, which so severely affected prematurely advanced vegetative growth.

Temperature conditions prevalent at this time, as well as those for the entire month of March, are shown in table 1, and are those recorded by the University of Illinois climatological station in Urbana, in conjunction with the U. S. Weather Bureau (1932).

TABLE 1. *Daily temperature extremes for March, 1932 (Champaign and Urbana vicinity)*

Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Maximum	51	46	43	47	44	16	18	15	21	25	28	25	33	28	41	60
Minimum	38	36	38	35	15	6	5	8	5	6	12	15	13	12	18	30
Day		17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Maximum		51	47	50	38	33	35	39	53	66	58	46	56	69	61	44
Minimum		33	24	31	24	30	24	25	25	42	40	33	33	35	40	30

Since changes in growth processes and activities of plants are conditioned and affected more by extreme temperature fluctuations than by mean or average temperatures, emphasis must necessarily be placed upon these extremes and, in the case of winter injury, particularly upon the lower extremes. Hence, the sudden drop to 15° F. March 5 and the low temperatures prevalent during the three days thereafter, which were never higher than 18° F., evidently occasioned and were chiefly responsible for injury to vegetation. Further, until March 15 the maximum temperature was above freezing only once, when, March 13, a temperature just one degree above freezing occurred. Minimum temperatures were, with one exception, constantly below freezing until March 25. On March 17, a minimum temperature of 33° F. was recorded.

GENERAL SYMPTOMS OF WINTER INJURY

The external symptoms indicative of winter injury manifested, in general, much similarity among the various kinds of trees and shrubs affected, although the extent or degree of injury differed in accordance with the particular species of plant.

Most conspicuous of the symptoms, and one which readily distinguished winter-injured plants from those not affected, was the presence of numerous scattered twigs entirely devoid of foliage, protruding from the tops of the plants or at the extremities of lateral branches in the lower portions. Often, too, portions of large branches presented the same leafless appearance. In such instances the young terminal twigs and the larger branches were dead, and as shown in figures 1 and 3 (pl. 1), they contrasted sharply with the leafy, uninjured portions of the plants. The extent of dying back as a result of winter killing varied from only a few inches to as much as several feet.

In figures 2 and 4 (pl. 1), in which are pictured the dead terminal portions of single branches of elm and pussy willow, another common and striking characteristic of winter injury is shown. This is the presence of much enlarged or partially opened buds killed by freezing. These dead buds stood out very conspicuously on injured plants and afforded a fairly reliable means

for distinguishing between twigs killed by freezing and those dead as a result of fungus invasion after the plants leafed out. In figures 1 and 3 (pl. 1) the dead buds are also evident.

The extent to which development, or unfolding, of buds had occurred prior to winter injury varied greatly, both among the different kinds of plants and among individuals of the same species. Buds of the most precocious trees and shrubs were fully opened, or almost so, when freezing occurred. Plants characterized by a more retarded growth exhibited only enlarged or partially unfolded buds, and on other plants, in which the initiation of growth activities was still more delayed, the buds were killed, apparently, just as growth was beginning, since they exhibited only slight enlargement.

The production of a dense, compact type of foliar growth was another characteristic exhibited by some winter-injured plants, but this occurred only as an accompaniment of severe injury. This characteristic will be discussed further in the descriptions of injury to particular plants.

Injury to elm. The American elm (*Ulmus americana* L.) normally flowers and begins growth activities earlier than most trees in the Champaign and Urbana vicinity and, consequently, was commonly injured. Both parkway and lawn elms were equally affected, and injury varied from slight, consisting of only a few, scattered, killed twigs, to very severe, as shown in figure 1 (pl. 1). This elm, growing in Carle Park in Urbana, had, almost without exception, the ends of all terminal and lateral branches killed. The degree of injury ranged from small dead twigs three to six inches in length to larger branches dead four to six feet back.

Another character exhibited by this tree, and one which has just been referred to in the discussion of general winter injury symptoms, was the development of very dense foliage below the injured parts. This compact leafy growth was produced as a result of the development of lateral buds which normally would have remained dormant until the next growth period in the following spring. Developing at the bases of already expanded leaves, these scattered young shoots produced at first small tufts of leaves. Later these developed further into a more or less continuous, thick foliar growth along the branches, which, intermingled with the numerous dead twigs and branches, imparted to the tree the appearance of tangled growth.

While the number of elms suffering winter injury was quite large, instances in which injury was as severe as that shown in figure 1 (pl. 1) were relatively infrequent. Injury to elm consisted mainly in the killing back of terminal and lateral branches for distances of only eight to fourteen inches from the tip.

Injury to pussy willow. With very few exceptions, the cultivated pussy willow (*Salix Caprea* L.) experienced winter injury which, in most instances, was very severe. General occurrence of such injury would be expected, however, in view of the fact that the pussy willow was the first shrub to flower in the Champaign and Urbana locality, and consequently growth processes were quite active at the time freezing occurred.

Injury to pussy willow was conspicuously evident among terminal shoots in the tops of the plants, though it also occurred on some lateral branches as well. As shown in figure 3 (pl. 1), the leafless portions of terminal branches contrast markedly with the lower leafy portions of the plant, particularly because of the large, swollen flower buds present on the injured parts.

In figure 4 (pl. 1), which shows a portion of an injured terminal branch of pussy willow, it is to be noted that almost all of the flower buds were considerably expanded when injury occurred. A few, however, were only partially open, and the terminal and the first sub-terminal buds appear to have been killed before opening. This is typical of the appearance of winter-injured pussy willows throughout the Champaign and Urbana vicinity. The extent of winter injury to pussy willow ranged from the killing of two- or three-inch portions of branches to those dead for a distance of four feet back.

Injury to other plants. Elm and pussy willow were by no means the only plants in the Champaign and Urbana vicinity in which winter injury occurred. Among the other trees and shrubs most commonly and severely injured were poplar and privet.

Of the various poplars grown for lawn and ornamental purposes, the Lombardy (*Populus nigra* L. var. *italica* Du Roi) appeared to be more extensively injured than the Carolina (*Populus canadensis* Moench.), White (*Populus alba* L.), or Simon poplar (*Populus Simonii* Carr.). This was partly due to the more extensive use and general cultivation of the Lombardy poplar but more especially to the fact that plantings of this poplar are usually single or in rows quite isolated from other trees and are thus often left quite unsheltered.

Winter injury also occurred in various species of privet but was more noticeable in the common privet (*Ligustrum vulgare* L.), owing to its more general use as a cultivated ornamental.

The character of winter injury to poplar and privet was quite similar to that which has already been described for elm and pussy willow in that the extremities of both terminal and lateral branches were killed. As much as six-foot portions of terminal branches were killed in poplar, and in privet the larger branches were frequently killed two or three feet back.

Most noticeable of the various trees and shrubs injured to a lesser extent was the hard maple (*Acer saccharum* Marsh.) Numerous small twigs were blighted in the tops of these trees, but in no observed instance was injury so severe as that exhibited by elm, pussy willow, poplar, or privet.

As a general consideration of the various plants affected by winter injury, it may be said that any species of plant which had initiated growth activities prior to the time when freezing occurred was subject to injury, so that if a complete list of all plants so injured were available, a very large number of species would undoubtedly be included.

HISTOLOGICAL CHARACTER OF WINTER INJURY

Numerous articles pertaining to winter injury of various plants exist, but only a relatively small number of them treat even briefly of the histological changes that result from freezing or low temperature conditions.

Review of literature

The most extensive study of the histological aspects of winter injury, and especially of frost ring formation, is that of Rhoads (1923), who investigated the pathological anatomy of late frost injury as exhibited by various conifers in northern Idaho, northeastern Washington, northwestern Montana, and (supplemented later by other material) in Washington, D. C., and Missouri. Among the conifers studied were *Pinus albicaulis* Engelm., *P. contorta* Dougl., *P. densiflora* Sieb. & Zucc., *P. lambertiana* Dougl., *P. monticola* Dougl. ex Lamb., *P. ponderosa* Dougl., *Picea Engelmanni* (Parry) Engelm., *Larix occidentalis* Nutt., *Pseudotsuga taxifolia* (Lam.) Britton, *Abies grandis* Lindl., *A. lasiocarpa* (Hook) Nutt., *Tsuga heterophylla* (Raf.) Sarg., *T. mertensiana* (Bong.) Carr., *Thuja plicata* D. Don., *Chamaecyparis lawsoniana* (Murr.) Parl., *Sequoia gigantea* DC., and *Taxus baccata* L.

The few earlier investigations concerning the histological character of winter injury have been referred to and adequately summarized by Rhoads, and a repetitional review of them is unnecessary. Moreover, since the appearance of Rhoads' investigations, very few additional investigations have treated of the histological character of winter injury in other plants.

Wood (1929) reported injury of currant and sultana vines at Renmark, Berri, Monash, and Barmera in South Australia, and at Mildura and Red Cliffs in Victoria, due to a shallow drift of cold air which swept over irrigation areas of the Murray valley in September, 1927.

Stem tissue of the young currant and sultana shoots revealed a killing and disintegration of cortical, medullary ray, and pith cells, as well as torsion and laceration of woody tissue. Older portions of the vines evidenced cambial degeneration as a first effect of the low temperatures. Gradual degeneration of cortical and phloem tissue then succeeded cambial necrosis. Following the breakdown of these outer tissues, air was admitted to tracheae and tracheids, rendering them functionless. This separation of outer and vascular tissue also caused the outermost pith cells to degenerate. Pith cells adjacent to those which were peripheral, however, became meristematic and formed cells with cutinized walls, thus temporarily protecting the living pith cells from further disintegration. After separation from the vascular elements, however, they, too, eventually died and disintegrated.

Bittman (1930) described the formation of a false heartwood in red beech, which was occasioned by frost injury during the severe winter of 1928-1929 in the Russian Carpathians, Galicia, and Czechoslovakia.

This false heartwood differed from true heartwood in that the tracheal

vessels remained functional and no tyloses were formed in them. Formation of the false heartwood apparently resulted from obstruction of the transpirational stream, the latter being due to extensive killing of superficial tissues at the base of branches where meristems commonly occur in the beech. Accordingly, this caused a higher water content to occur in the inner than in the outer portion of the sapwood.

Materials and methods

For determining the histological changes in specimens from some of the winter-injured trees and shrubs, it was desirable to obtain portions of stems which had been injured but not killed by freezing, so that subsequent growth had occurred. Such material would occur immediately below the winter-killed parts and, accordingly, three- or four-inch portions of the still living stems were taken from such points and preserved in formalin-acetic-alcohol solution until sectioned.¹ Microtome sections of the stems were cut at 12 to 18 μ and stained with Pianezze III, B. This stain was employed in order to afford assurance that no fungi were present in the stem and that all histological changes observed could be attributed entirely to the effects of winter injury. The adequacy of this stain for general wood structure was also recognized inasmuch as it stains deeply and delineates clearly the primary walls of xylem tissue.

Histological character of winter injury in Ligustrum

Of the various specimens secured from winter-injured plants in the vicinity of Champaign and Urbana, those from *Ligustrum* proved most suitable for histological study. In this material several histological changes occurred, some of which were exhibited only to a slight extent or were entirely lacking in specimens from other plants. Consequently, the study of frost ring formation in *Ligustrum* is presented most fully and that of other plants is given only comparative treatment.

Frost rings. In addition to the external symptoms already described as indicative of winter injury, various internal symptoms may also be evident when living portions of stems immediately below the winter-killed parts are examined. Often, when a transverse cut is made through that portion of the stem, a narrow brown ring, or frost ring, as it is termed, is revealed in the wood.

In *Ligustrum* the frost ring was very narrow and a tangential cut showed it to be continuous through the stem for a considerable distance. At first glance it might be readily assumed that such wood discoloration indicated fungous parasitization of the stem. The regularity and the even, clear-cut delineation of the frost ring discoloration are to be distinguished, however,

¹ The solution used was made up as follows: 50 per cent alcohol, 90 cc.; commercial formalin, 5 cc.; glacial acetic acid, 5 cc.

from the more irregular and diffused type of wood discoloration resulting from fungous invasion.

Frost rings similar to those in *Ligustrum* were also present in winter-injured specimens of *Ulmus*, *Salix*, and *Populus*. A frost ring in a two-year-old stem of *Ligustrum* is shown in figure 5 (pl. 2), though, as a result of magnification, the macroscopic regularity of the ring is not so apparent. Its position at the origin of the current year's growth ring indicates that it resulted from winter injury occurring shortly after growth began in the spring.

Microscopic examination shows this frost ring, in *Ligustrum*, to be the combined result of several histological alterations in the xylem tissue. In brief, five changes or conditions are to be noted in this respect, as follows: (1) *collapsed cells*; (2) *formation of parenchymatous wood*; (3) *gummosis*; (4) *formation of traumatic parenchymatous tissue*; and (5) *widening of the xylem rays*. A discussion of each of these characters follows.

Collapsed cells. The frost ring proper is composed of numerous crushed or collapsed cells which have been compacted exceedingly as a result of ice formation in the stem. The general features of this condition are shown in figure 6 (pl. 2). Here the walls of the compressed cells are still visible, but their normal arrangement in more or less regular radial rows is so disrupted that they appear as a very distorted and irregular cellular mass.

In figure 7 (pl. 2), which shows a portion of the frost ring protruding irregularly into the wood, the distorted condition of the walls of the deranged and crushed cells may be seen to still greater advantage. These crushed cells constitute early wood tissue that was formed prior to the occurrence of winter injury. They are much more extensive in *Ligustrum* than in any of the instances described and illustrated by Rhoads, who also noted a compacting of the young cambial cells in various conifers to the extent that their outlines became more or less indistinguishable. Rhoads also observed that the thick-walled but unligified cells frequently collapsed to the extent that their walls became crumpled but not entirely crushed. In *Ligustrum* the crushed cells indicate that they, too, were still unligified when freezing occurred, inasmuch as they were subsequently crushed by the ice mantle formed between the wood and bark when freezing occurred.

At some points the frost ring in *Ligustrum* has a diameter corresponding to the width of not more than two or three crushed cells; at others it is ten to twelve cells wide. Only a slight crushing and crumpling of cells similar to that described for *Ligustrum* was noted in *Ulmus* and *Populus*, and it was entirely unobserved in *Salix*.

Parenchymatous wood. The formation of a zone of parenchymatous wood² upon resumption of growth is a characteristic of winter injury which

² *Parenchymatous wood* (previously denoted as *parenchyma wood* by Rhoads and other investigators of frost ring formation) is not to be regarded as synonymous with *wood parenchyma*, which is of normal and frequent occurrence in the xylem tissue of most woody plants. Inasmuch as normal *wood parenchyma* is also referred to occasion-

has been noted by several investigators. Rhoads states that the interpolation of the zone of parenchymatous wood observed during his investigations apparently resulted from a transitory weakening of the compressing influence exerted by the bark girdle on the cambium due to the disrupting action produced by freezing. Thus, the cambium responded to this decreased constriction of bark by forming large parenchymatous cells until in its further growth it was again so restrained by the bark girdle that formation of normal wood elements reoccurred.

Formation of parenchymatous wood was one of the most conspicuous histological changes induced by winter injury in *Ligustrum*, and as shown in figure 6 (pl. 2), this zone of parenchymatous wood occurs immediately peripheral to the collapsed xylem tissue.

The parenchymatous cells comprising this tissue vary much in size and shape as compared with normal wood parenchymatous cells and usually possess a diameter three to four times that of the latter. They may be elongated or nearly isodiametric. Their cytoplasmic contents are densely granular and frequently contain numerous large vacuoles. The nuclei are particularly large and prominent so that, occasionally in the smaller cells, the nucleus nearly bridges the opposite walls. The nuclei are usually spherical, but their shape may also vary to ovoid, ellipsoid, or irregularly elongate and flattened. They usually occupy a central position in the parenchyma cells but may also be appressed closely to the cell wall. The relative size and shape of the cells comprising the parenchymatous wood, as well as their cytoplasmic character and large nuclei, are shown in figure 7 (pl. 2), and to some advantage in the lesser magnification of figure 6 (pl. 2).

While the presence of this parenchymatous wood was conspicuous in the stem, its extent was not sharply delineated, since it merged irregularly into the normal and later formed wood by a gradual decrease of cell size, making the point of mergence barely perceptible. In *Ligustrum* this zone of parenchymatous wood was usually only six to eight cells wide, but frequently became as much as 12 to 16 cells wide before normal wood formation occurred.

In *Populus* the cells of the parenchymatous wood constituted the basic structure of the frost ring. As in *Ligustrum*, the thickness of the parenchymatous wood zone varied so that at some points its continuity was maintained only by two or three small cells, while at other intervals it was 14 to 16 cells wide. Parenchymatous wood was also present in winter-injured stems of *Ulmus* and *Salix*, though its occurrence in the observed instances was less extensive. Neither were the nuclei in the parenchymatous wood cells of *Ulmus*, *Salix*, and *Populus* so conspicuous and large as in *Ligustrum*. An especially noticeable feature in connection with all observed instances of parenchymatous wood formation was the abundant deposition of starch material in

ally as *parenchyma wood*, it is deemed advisable to term that parenchymatous tissue which forms abnormally in the wood as a result of mechanical injury such as freezing, *parenchymatous wood* rather than *parenchyma wood*.

this region. Many of the parenchymatous wood cells were completely filled with the large starch grains.

Gummosis. Observation of the *Ligustrum* frost ring under low magnification indicated that its brown coloration was to be ascribed to a discoloration of the cell walls. Greater magnification and more thorough observation revealed, however, that the cell walls, usually colorless, were only slightly discolored at the most and that the frost ring coloration was due, instead, to the brown cellular contents of the collapsed xylem tissue. This brownish substance within the cells was always quite dense, and though it often appeared smooth and homogeneous, it usually possessed a decided granular appearance.

This condition of gummosis is frequently a prominent manifestation of both mechanical and fungous injury of woody tissue, and in the case of *Populus* and *Salix* it afforded one of the most conspicuous features to be noted in conjunction with winter injury. Gummosis was also present in winter-injured specimens of *Ulmus* but to a lesser extent and occurred only infrequently.

The severe type of gummosis occurring in *Populus* is shown in figure 8 (pl. 2). This condition differs from that observed in *Ligustrum* in that gummosis occurs in the large cells comprising the parenchymatous wood. In *Ligustrum* it was entirely unobserved in the zone of parenchymatous wood and was found occurring only within the collapsed xylem tissue. In both *Ulmus* and *Salix* gummosis also occurred in the parenchymatous wood and in the latter sometimes intercellularly. Rhoads also noted the occurrence of gummosis in the parenchymatous wood of conifers injured by late frost.

Traumatic parenchymatous tissue. At various points, as shown in figure 5 (pl. 2), portions of the *Ligustrum* frost ring protrude very irregularly into the current year's wood. This condition may also be seen at different magnifications in figures 7, 9, and 10 (pl. 2).

These irregular projections of the frost ring appear to have been produced by thawing of the stem tissue, which resulted in the formation of numerous radial clefts extending outward from the point of origin of the current year's xylem and also in the limited amount of xylem tissue which had been formed prior to freezing. Rhoads observed similar radial clefts in the winter-injured stems of conifers and attributed their formation to a preponderance of tangential contraction over radial contraction accompanying freezing and subsequent thawing of the stem tissue.

In the case of *Ligustrum* these schizogenous cavities did not remain as empty lacunae but were subsequently filled with new parenchymatous tissue, as shown in figures 7 and 9, and less prominently in figures 5 and 10 (pl. 2). This traumatic parenchymatous tissue may consequently be regarded as an inner callus tissue.

The morphological structure of the traumatic cells is quite similar to that of cells comprising the parenchymatous wood. They possess large, prominent nuclei and vacuolated granular cytoplasm. An abundant deposition of

starch granules also occurs in these cells. The shape and size of the traumatic parenchymatous cells are quite varied, since they conform to the irregularities and sizes of the cavities they fill. Thus, in figure 7 (pl. 2) it is shown that the two small cavities have been filled, each by a single, large parenchymatous cell, while in figure 9 (pl. 2) it is seen that several cells were necessary to fill a larger cavity.

In many instances it is clearly discernible that these traumatic parenchymatous cells were formed by lateral division of xylem ray cells. In other instances the cavities were apparently filled with cells arising either from the cambium itself or by division of the cells comprising the zone of parenchymatous wood. The occurrence of radial clefts was not observed in any of the winter-injured specimens of *Ulmus*, *Salix*, and *Populus* examined.

Widening of the xylem rays. Rhoads observed that lateral expansion and displacement of the xylem rays were by far the most conspicuous and characteristic feature to be noted in late-frost injury of conifers. The rays were apparently stimulated to lateral broadening by diminution of the pressure normally exerted by the young wood elements. He attributed the lateral displacement to the fact that the cells constituting the rays were stretched during ice formation within the stem and that this condition remained after thawing.

While increased width, or lateral expansion, of the xylem rays was also a conspicuous histological change accompanying winter injury of *Ligustrum*, only slight displacement of the rays was seen to have occurred and then only in a few such instances. Normally, the xylem rays of *Ligustrum* are either one or, more frequently, two cells in width. Those portions of the rays extending through and beyond the frost ring, however, as shown in figure 10 (pl. 2), are from three to six cells wide. In addition to the increase due to the number of cells, there was noted, also, an accompanying increase of cell size, so that the widened xylem rays may be considered to exhibit both hypertrophy and hyperplasia as a result of winter injury.

In general, cells of widened xylem rays are considerably shorter than cells comprising normal rays. The elongated type of cell characterizing the latter is of rather infrequent occurrence in the widened rays whose shorter cells are indicative of the more frequent occurrence of cell division. Near the completion of the growth season, however, the xylem rays had again attained an almost normal diameter as a result of diminution in both cell size and cell number.

Lateral expansion of xylem rays also occurred in *Ulmus*, *Salix*, and *Populus*. The normal uniseriate xylem rays of *Populus* and *Salix* were converted to a biseriate condition in the former and to either a bi- or triseriate condition in the latter. In *Ulmus* the xylem rays were frequently observed to be four cells wide at some points, as compared with the normal uni- or biseriate condition.

SUMMARY

In early March, 1932, the general occurrence of freezing temperatures in Illinois, following a winter of exceptional mildness, resulted in extensive and severe winter injury to a number of trees and shrubs. In the vicinity of Champaign and Urbana, injury was most commonly manifested by elm, pussy willow, poplar, privet, and maple, though a number of other plants were also injured.

The general character of winter injury was quite similar in its manifestation among the various trees and shrubs. Most conspicuous of the general symptoms was the killing of small twigs and terminal portions of branches, chiefly in the topmost parts of the plant. In some instances the production of a dense, compact foliar growth due to the development of normally dormant buds was an accompanying characteristic of winter injury.

The extensiveness and severity of injury varied considerably in accordance with the species or individuality of the plant, and its situation with respect to shelter, but was determined chiefly by the degree of progress attained in growth activities prior to the occurrence of freezing temperatures.

Frost rings were exhibited by all winter-injured plants examined. A histological study of frost ring anatomy in *Ligustrum*, with a comparative study of frost rings in *Ulmus*, *Salix*, and *Populus*, revealed five histological changes as a result of winter injury. These were: (1) collapsed cells, (2) formation of parenchymatous wood, (3) gummosis, (4) formation of traumatic parenchymatous tissue, and (5) widening of the xylem rays. Winter injury to woody dicotyledonous plants is shown to correspond in all important particulars to that already described for conifers.

The writer is indebted to Dr. L. R. Tehon, botanist of the Illinois State Natural History Survey, for suggesting the desirability of this study. Acknowledgment is also due Mr. Roy R. Hamm, photographer of the University of Illinois, for the photographic illustrations.

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URBANA, ILLINOIS

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EXPLANATION OF PLATES

PLATE I

Fig. 1. An American elm in Carle Park, Urbana, showing a severe type of winter injury. Numerous winter-killed twigs and branches occur throughout the tree but are most abundant in the upper portions. Photographed June 14, 1932.

Fig. 2. The terminal portion of a branch of American elm, showing winter-killed twigs and some of the still-persistent, open flower buds. Photographed June 2, 1932. $\times 0.11$.

Fig. 3. Pussy willow exhibiting severe winter injury typical of that which occurred in the Champaign and Urbana vicinity. Note the conspicuous, still-persistent winter-killed buds on the terminal shoots. Photographed June 14, 1932.

Fig. 4. A terminal branch of pussy willow which suffered winter injury. The entire flowering branch was killed, as well as both the expanded and unexpanded flower buds. Notice also that the terminal buds were killed before opening. Photographed June 2, 1932. $\times 0.09$.

PLATE 2

Fig. 5. Photomicrograph of a transverse section through a two-year-old stem of *Ligustrum*, showing the frost ring at the origin of the annual growth ring of the current season. $\times 13$.

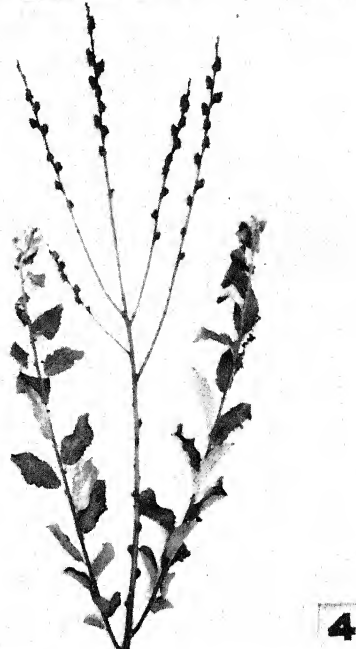
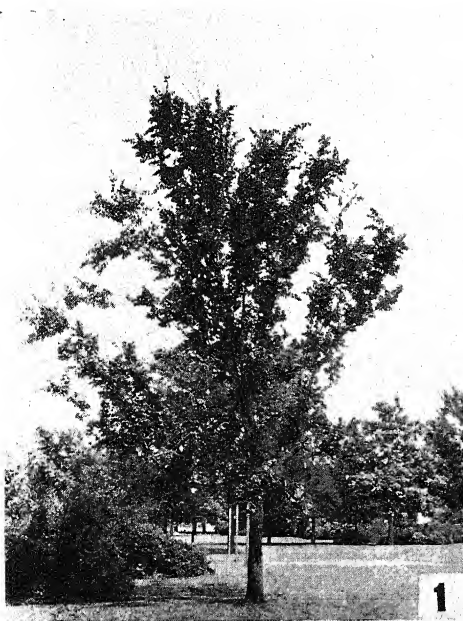
Fig. 6. Photomicrograph of the *Ligustrum* frost ring, depicting the early formed xylem tissue which has been greatly crushed and compacted by freezing so as to form the structure of the frost ring proper. The formation of a zone of parenchymatous wood has also occurred peripheral to the frost ring. $\times 285$.

Fig. 7. Photomicrograph of a portion of the *Ligustrum* frost ring, depicting the crushed and greatly deranged condition of the xylem tissue comprising the frost ring. Notice also the large traumatic parenchymatous cells filling the radial clefts. The cytoplasmic contents and large nuclei of the parenchymatous wood cells are also quite prominent. $\times 635$.

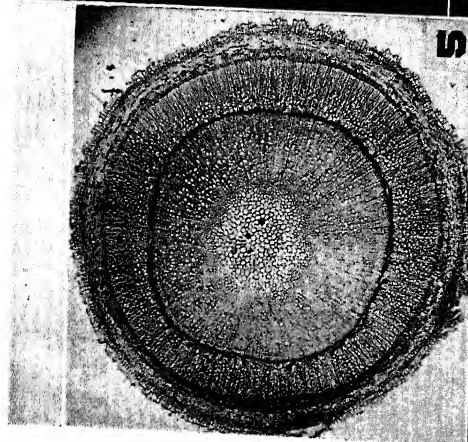
Fig. 8. Photomicrograph showing an extremely severe type of gummosis in the region of frost ring formation in *Populus*. $\times 285$.

Fig. 9. Photomicrograph showing a radial cleft in the *Ligustrum* frost ring filled with traumatic parenchymatous tissue. $\times 160$.

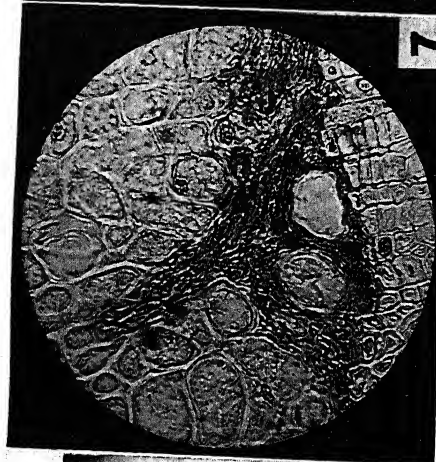
Fig. 10. Photomicrograph of a portion of the frost ring illustrated in figure 5, showing the irregular radial splitting which occurred in the xylem tissue as a result of freezing and also the great increase in width of the xylem rays following the period of injury. $\times 64$.



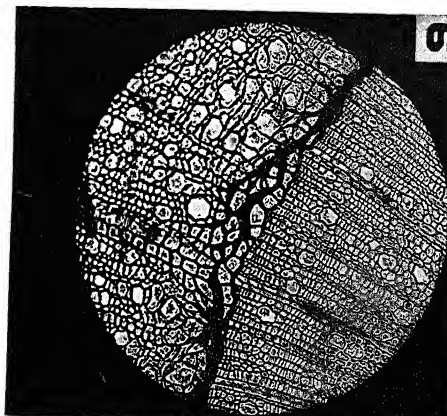
HARRIS: FROST RING



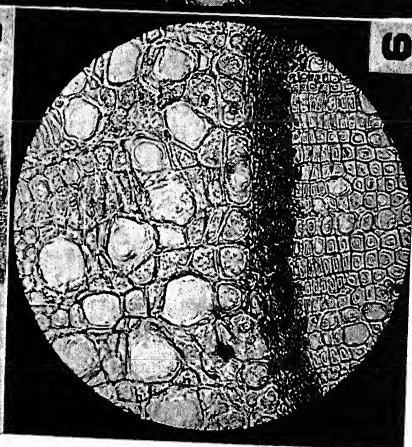
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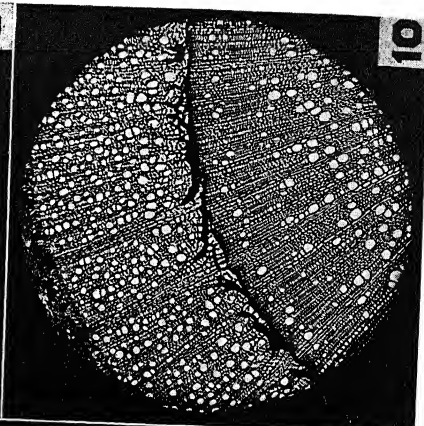
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10

HARRIS: FROST RING

THE ALGAL GENUS *TETRASTRUM*¹

ELBERT H. AHLSTROM AND LEWIS H. TIFFANY

(Received for publication April 15, 1933)

This paper is an attempt at a critical evaluation of the species of the genus *Tetrastrum*. The variations in form, in numbers of spines, and in size of the coenobia in the species are considered in some detail. Some previously described species are referred to separate genera, while others are shown to be synonyms.

There appears to be a real need for a critical examination and evaluation of the species of many genera of the Chlorococcales. Species are too often described as new by workers who do not have access to the literature on this algal group. Such species are frequently found to be synonymous with previously described forms, particularly when a serious attempt is made to analyze the variations a species presents in the same and varying habitats.

No attempt is made here to record variations in growth in pure cultures. The species of the genus are considered entirely from collections made in the field, many hundred observations having been made in the last few years. For the few species not found in our collections, we have had to depend upon descriptions from the literature.

The genus *Tetrastrum* was established by Chodat in 1895 to include the species, *Staurogenia heteracantha* Nordstedt, to which he later added *St. multiseta* Schmidle. In 1897 Schroeder established the genus *Cohniella* for *C. staurogeniaeformis*, which was in 1900 referred to *Tetrastrum* (*T. staurogeniaeforme*) by Lemmermann. In the same year Schmidle placed *Tetrastrum* as a subgenus under *Staurogenia* with the following species: *St. schroederi* (= *Cohniella staurogeniaeformis*), *St. multiseta*, *St. multiseta* var. *punctata*, *St. apiculata*, *St. heteracantha*, *St. alpina*. Brunnthaler in Pascher's "Süsswasserflora" (1915) recognized *Tetrastrum* and included in it *Crucigenia tetracantha* G. S. West. Playfair (1916) added *T. elegans*; Printz (1927) referred *Crucigenia truncata* G. M. Smith to *Tetrastrum*; and Griffiths (1927) described *T. rocklandiensis*.

The genus has a wide geographical distribution. Some of the species are very common in certain habitats and singularly absent from others. The genus is particularly abundant, for example, in Buckeye Lake and Maumee River in Ohio, but in over a hundred samples of plankton algae from Florida not a single specimen has been so far observed.

¹ Papers from the Department of Botany, Ohio State University, No. 324.

TETRASTRUM

CHODAT 1895

Bull. Herb. Boiss. 3(1): 114, 1895; Brunnthaler in Pascher Süßwasserfl. Deut. Oest. u. d. Schw. 5: 176, 1915; G. M. Smith Phytopl. Inland Lakes Wisc. p. 145, 1920; Printz in Engler and Prantl Die Nat. Pflanzenfam. 3: 148, 1927.

Coenobia always 4-celled, with cells cruciately arranged in a flat plate, with or without a small open space at the center of colony, at times embedded in a thin gelatinous envelope. Cells angularly rounded, or ovoid, broadly triangular or semicircular; usually with one or more setae or short spines, or papillae, on the free face. Chloroplast single, laminate, with or without a pyrenoid; chloroplast dividing into 4 previous to autocolony formation. Multiplication by autocolony formation in any cell, the four cells of the coenobe generally producing autocolonies simultaneously. Plankton.

Tetrastrum, closely related to *Crucigenia*, has two markedly differentiating characters: the presence of spines on the margins of the cells (except in *T. glabrum*), and the four-celled nature of the colony (except during autocolony formation) which is never associated in multiple coenobia. The genus belongs to Class Chlorophyceae, Order Chlorococcales, and Family Scenedesmeaceae.

Type species: *Tetrastrum heteracanthum*.

KEY TO THE SPECIES

1. Coenobia 8-sided, cells semicircular1. *T. alpinum*
1. Coenobia not 8-sided 2
 2. Free face of cell with spines 3
 2. Free face of cell with punctae5. *T. punctatum*
 2. Free face of cell smooth4. *T. glabrum*
3. With several spines usually of the same length 5
3. With 1 or 2 spines; if 2, one spine much shorter 4
 4. Longer spine up to 24μ in length3. *T. heteracanthum*
 4. Longer spine up to 46μ in length3a. *T. heteracanthum* var. *longispinum*
5. Spines 3- 10μ long2. *T. staurogeniaeforme*
5. Spines 18- 23μ long2a. *T. staurogeniaeforme* var. *longispinum*

TREATMENT OF SPECIES

1. TETRASTRUM ALPINUM Schmidle, Fig. 35-36

Ber. Deut. Bot. Ges. 18: 157, pl. 65, fig. 24, 25, 1900. *Crucigenia quadrata* var. *octogona* Schmidle Oesterr. bot. Zeitschrift 1895.

Coenobia 10- 14μ in diameter, 4-celled, 8-sided, with a rather large open space in the center. Cells 4- 6μ in diameter, semicircular, the free face of cell flat and papillate. Chloroplast single, filling entire cell, with a pyrenoid.

Reported by Schmidle from Davoser Sea, Switzerland; and from Altrhein near Roxheim, Germany.

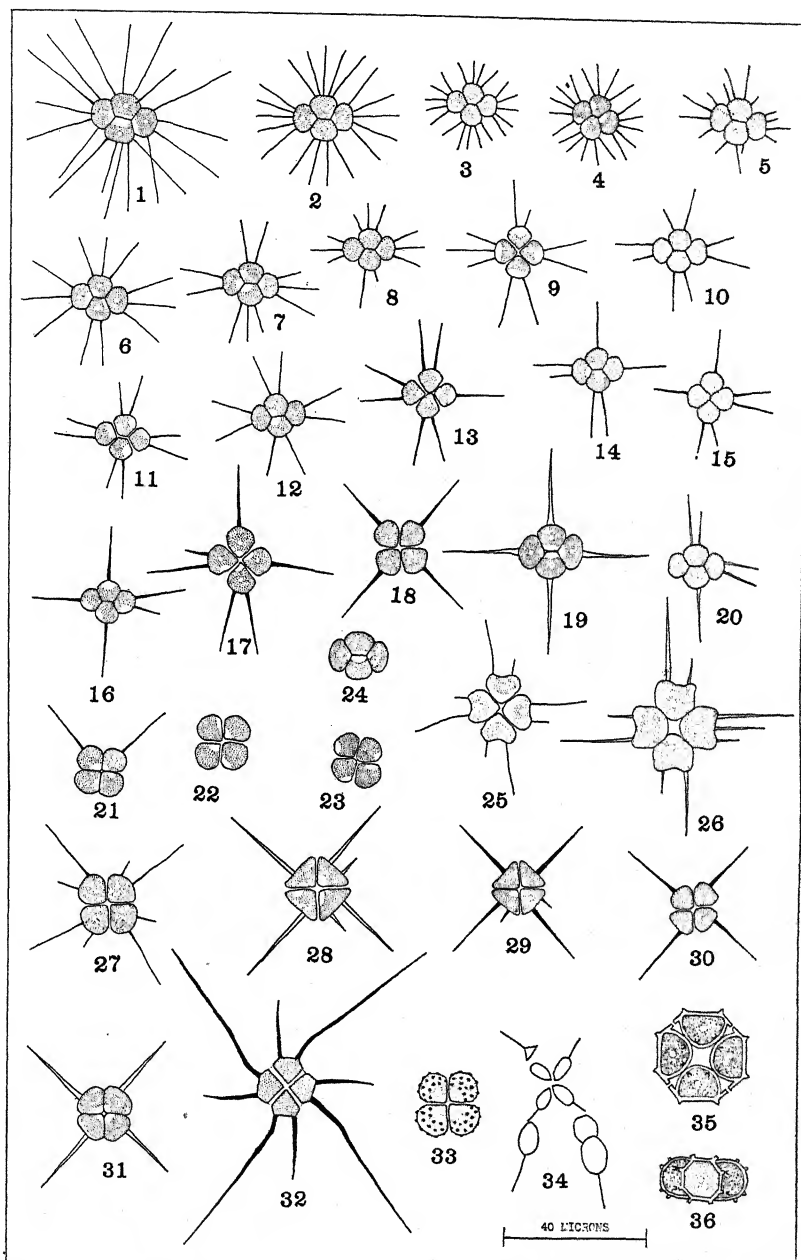


Fig. 1. *Tetrastrum staurogeniaeforme* var. *longispinum* (after G. M. Smith). Fig. 2-5. *T. staurogeniaeforme*. Fig. 6-21. Series showing gradual reduction in number of spines in *T. staurogeniaeforme* through "elegans" form and approaching *T. glabrum*. Fig. 22-24. *T. glabrum*. Fig. 25-27. *T. heteracanthum*. Fig. 28-31. Series showing gradual reduction in number of spines in *T. heteracanthum* to the "elegans" form. Fig. 32. *T. heteracanthum* var. *longispinum* (redrawn from Schiller). Fig. 33. *T.*

This is a very distinct species and our treatment of it is based entirely on Schmidle's description and figures. It differs markedly from other members of the genus both in shape and in the type and position of the papillae.

2. *TETRASTRUM STAUROGENIAEFORME* (Schroeder) Lemmermann, Fig. 2-5

Ber. Deut. Bot. Ges. 18: 95, 1900; G. M. Smith, Phytoplank. Inland Lakes Wisc. p. 149, pl. 37, fig. 5-6, 1920; *Cohniella staurogeniaeformis* Schroeder, Ber. Deut. Bot. Ges. 15: 373, pl. 17, fig. 5, 1897; *Tetrastrum heteracanthum* var. *homoiacanthum* Huber-Pestalozzi, Archiv Hydrobiol. 20: 423, text fig. 1, 1929; *Tetrastrum elegans*, Playfair (in part), Proc. Linn. Soc. N. S. Wales 41: 832, pl. 57, fig. 6, 1916; *Crucigenia hastifera* Arnoldi (in part), Zeitschr. Russ. Bot. Ges. 7: (1922) 1924; *Staurogenia* (*Tetrastrum*) *schroederi* Schmidle, Ber. Deut. Bot. Ges. 18: 156, 1900.

Coenobia (without spines) 7-15 μ in diameter, 4-celled, with or without (usually without) a minute open space at the center of the colony, embedded in a very delicate gelatinous envelope. Cells 3-6 μ in diameter, angularly rounded or oval to broadly triangular, with 1-8 usually delicate spines on the free faces; spines 3-10 μ long. Chloroplast single, laminate, parietal, with or without a pyrenoid.

Localities found: Ohio: Buckeye Lake; Terwilliger's Pond on S. Bass Island (Lake Erie); Mirror Lake of University Campus, Columbus; Maumee River; Ottawa River; Portage River. Iowa: Miller's Bay (Okoboji Region). California: Echo Park (Los Angeles). N. Carolina. Latvia.

Previous American records: Wisconsin (G. M. Smith, 1920), Iowa (G. M. Smith, 1926; Prescott, 1931), Indiana (B. H. Smith, 1932).

2a. *TETRASTRUM STAUROGENIAEFORME* var. *LONGISPINUM* G. M. Smith,
Fig. 1

Trans. Am. Micro. Soc. 45: 186, pl. 15, fig. 10, 1926.

Cells somewhat larger than in type, with the spines nearly twice the diameter of the colony. Coenobia (without spines) 11-15 μ in diameter; spines 18-23 μ long.

Localities observed: Very rare in Maumee River.

Previous records: Iowa (G. M. Smith, 1926).

Easily distinguished by its very long spines. It is probably an ecological variety.

3. *TETRASTRUM HETERACANTHUM* (Nordstedt) Chodat, Fig. 25-27

Bull. Herb. Boiss. 3: 113, 1895; G. M. Smith, Trans. Amer. Micro. Soc. 45(3): 187, pl. 15, fig. 16-20, 1926; Tiffany and Ahlstrom, Ohio Jour. Sci. 31(6): 464, pl. 3, fig. 38-40, 1931; *Staurogenia heteracantha* Nordstedt, Bot. Not. 1882: 56, text-fig. A-B, 1882; Schmidle, Ber. Deut. Bot. Ges. 18: 157, pl. 6, fig. 15-20, 1900; *Crucigenia heteracantha* Wlozynska, Hedwigia 55: 200,

pl. 7, fig. 10, 1914; *Tetrastrum elegans* Playfair (in part), Proc. Linn. Soc. N. S. Wales 41: 832, pl. 57, fig. 6, 1916; *T. elegans* var. *dentatum* Playfair, *ibid.* 41: 833, pl. 47, fig. 7, 1916; *Crucigenia hastifera* Arnoldi (in part), Zeitschr. Russ. Bot. Ges. 7: (1922) 1924. *T. heteracanthum* var. *minor* Roil, Archiv Russes de Protistol. 4: 150, pl. 6, fig. 40, 1925; *ibid.* 5: 37, pl. 1, fig. 12, 1926.

Coenobia 7–23 μ in diameter, 4-celled, usually axial in arrangement of cells, with or without a minute open space at the center of the colony, embedded in a very delicate gelatinous envelope; thickness of envelope usually about half the diameter of the cells. Cells (without spines) 2–11.5 μ in diameter, broadly triangular to angularly rounded, with 1–2 spines (usually 2) protruding through the gelatinous envelope; length of longer spines 8–24 μ , of shorter spines 1–9 μ ; when the 2 spines are present, one is markedly shorter and more delicate than the other. Chloroplast single, laminate, parietal, with or without pyrenoids.

Localities observed: Ohio: Buckeye Lake; Terwilliger Pond, Put-in-Bay, Lake Erie; ponds on Bass Islands; Mirror Lake, Ohio State University campus; Maumee River; Ottawa River. Michigan: Raisin River at Monroe.

Previous records: Iowa (G. M. Smith, 1926), Indiana (B. H. Smith, 1932), Sweden, Russia, England, France, Latvia, Africa, Australia.

The shorter spine in this species may be lacking on some or all cells of the coenobium, and the species may assume the *elegans* form (see later). The longer spine varies much in length and stoutness on different individuals—at times delicate and on other individuals stout. Out of several hundred specimens of *T. heteracanthum* observed, only two showed curved spines, the straight spine occurring almost with uniform regularity. The short spine usually alternates with the longer spine around the periphery of the colony, but even this arrangement is variable (see also G. M. Smith, Trans. Amer. Micros. Soc., 1926). The outer face of the cell varies considerably from the usually outwardly cordate shape to truncate or rounded or asymmetrically acute. As noted later, the axial form of the colony is the usual type in the species.

3a. TETRASTRUM HETERACANTHUM var. LONGISPINUM nov. var., Fig. 32
Tetrastrum heteracanthum Schiller Oesterr. Bot. Zeitsch. 73: 2, text-fig. 2, 1924.

Var. cum echinis longioribus; ceterum ut in typo.

Longer spine up to 46 μ in length; shorter spine up to 22 μ long; otherwise as in the species.

Locality: Austria (Schiller).

This long-spined form reported by Schiller seems worthy of varietal rank. Out of the numerous individuals of the species proper that we have observed, none have had spines more than half of the length reported by Schiller. This may be only an ecological variety.

4. *TETRASTRUM GLABRUM* (Roll) nov. comb., Fig. 22-24

Tetrastrum staurogeniaceforme var. *glabrum* Roll, Annales de Protistol. 1 (4): 165, fig. 8, 1928; *Crucigenia triangularis* Playfair (non Chodat) Trans. Linn. Soc. N. S. Wales 41: 57, fig. 4, 1916.

Coenobia 7-15 μ broad, 4-celled, with cells in either axial or cruciform arrangement (cruciform more common), with or without an open space in the center of the colony, embedded in a very delicate gelatinous envelope. Cells 3-7 μ broad, angularly rounded or oval to broadly triangular, without spines. Chloroplast single, laminate, parietal, with or without a pyrenoid.

Collected in Ohio from Maumee River and from Haunck Pond on Middle Bass Island in Lake Erie; previously reported from River Dnieper, U. S. S. R., and from Australia.

This species is quite similar to *Crucigenia quadrata* especially the form of the species that Chodat designated as *C. triangularis*. *T. glabrum* differs, however, in the absence of compound coenobia and in the often cruciform shape of the colony.

5. *TETRASTRUM PUNCTATUM* (Schmidle) nov. comb., Fig. 33

Tetrastrum multisetum var. *punctatum* (Schmidle) Brunthaler in Pascher Süsswasserfl. Deut. Oesterr. u. d. Schw. 5: 177, fig. 261, 1915; *Staurogenia multiseti* var. *punctata* Schmidle Ber. Deut. Bot. Ges. 18: 157, pl. 6, fig. 13-14, 1900; *Crucigenia quadrata* G. M. Smith Phytopl. Inland Lakes Wisc., p. 147, 1920.

Coenobia 8-12 μ in diameter, 4-celled, usually axial in arrangement of cells, with a minute open space at the center of the colony, embedded in a very delicate gelatinous envelope. Cells 3-6 μ in diameter, angularly narrowed to broadly triangular, with a number of small punctae confined mostly to the free faces of the cells. Chloroplast single, parietal, laminate, with a pyrenoid.

Localities observed: Stadt Kanal, Riga, Latvia.

Previous records: Davoser See (Switzerland). Russia.

This seems to be a valid species. G. M. Smith referred it to *Crucigenia quadrata*, observing a punctate form of the species which he took to be identical with Schmidle's variety. We have also observed this punctate variety of *C. quadrata* in Lake Michigan material. Collections of plankton algae kindly sent to us by Professor H. Skuja of the University of Riga (Latvia) contained specimens not identical with the punctate form of *C. quadrata*. This we have made a distinct species.

DISCUSSION OF SPECIES RELATIONSHIPS

Except for *T. alpinum* there is no sharp division among the species. Reference to fig. 2-31 will show a whole series of integrading forms among the various species. All of these figures were drawn from individuals obtained at one station near the mouth of the Maumee River (Toledo, Ohio). From this integrading series several interesting points are to be observed.

First, with regard to spines: (1) A graded series occurs from 5 spines per cell through 3, 2, 1, and finally reduced to no spine on some cells. (2) Individual cells of the same specimen differ in number of spines, some cells having 3, while others on the same specimen have but one. (3) Spines on the same individual are often of various sizes. (4) Reduction in number of spines is usually accompanied by an increase in stoutness of the spines present. (5) When there are two spines on a cell, one is often shorter and at times less stout than the other.

Second, with regard to shape: (1) *T. staurogeniaeforme* is typically longer than broad, the four cells of the colony not meeting in a common center. Out of 100 individuals observed, 95 showed this typical shape to a greater or lesser degree. This form of colony is designated, for convenience, "cruciform." (2) Individuals with few spines are associated with a colony form which is about as long as broad, and in which the 4 cells come together to a common center. This form of colony is termed "axial." (3) Typical specimens of *T. heteracanthum* are as long as broad with the four cells radiating from a common center. Of 40 individuals observed, 33 showed this type of colony. (4) Specimens with a single spine per cell (i.e., *T. elegans* Playfair) showed an equal number of each type of colony. Of 38 individuals observed for this character, 19 showed the cruciform type, 19 the axial. (5) The usual form of *T. staurogeniaeforme* is cruciform, and of *T. heteracanthum* is axial, but individuals of *T. staurogeniaeforme* may have the axial arrangement and individuals of *T. heteracanthum* may be cruciform.

The series (fig. 2-31) indicates that perhaps all these species intergrade. *T. elegans* might be formed either from a reduction in number of spines of *T. staurogeniaeforme*, or in the disappearance of the smaller spine of *T. heteracanthum* with a resulting migration of the remaining spine toward the center of the free face of the cells. Fig. 6-21 show variations of *T. staurogeniaeforme* leading to the "elegans" form, and fig. 28-31 indicate a rather complete series between *T. heteracanthum* and the "elegans" form. Since it cannot be recognized as a distinct species, and since its reduction to varietal rank would necessitate its placement under both *T. staurogeniaeforme* and *T. heteracanthum* (which would lead to complications), we feel justified in placing *T. elegans* in the synonymy of both species. *Crucigenia hastifera* Arnoldi (1922) is probably nothing more than the "elegans" form of these two species.

The intergrading of *T. staurogeniaeforme* into *T. heteracanthum* is suggested, but the series shown is neither complete nor convincing. A reduction in spines in both species to the spineless form is also suggested. Typical *T. heteracanthum* has characters which easily distinguish it from *T. staurogeniaeforme*. Even if it can be shown conclusively that the two intergrade it will perhaps be convenient to retain them as distinct forms.

T. glabrum probably results from a reduction in number of spines of

T. staurogeniacforme to the spineless form. But since it is the end product in the series, if such be the case, and since it perhaps represents the primitive form in this series, it seems best to regard it as a species. To regard it as a variety would mean the impossible assignment to both *T. heteracanthum* and *T. staurogeniacforme*.

SPECIES EXCLUDED FROM THE GENUS

1. *Tetrastrum anomalum* G. M. Smith, Trans. Amer. Micros. Soc. 45: 187, pl. 15, fig. 21-27, 1926.

Referred to the genus *Scenedesmus* as *S. anomalus* (G. M. Smith) Ahlstrom & Tiffany in Tiffany Contrib. Stone Laboratory, Ohio State Univ. 6: 69, pl. 12, fig. 298, 1934. Put-in-Bay, Ohio.

2. *Tetrastrum tetracanthum* (G. S. West) Brunthaler in Pascher Süßwasserfl. Deut. Oester. u. d. Schweiz 5: 175, fig. 263, 1915; *Crucigenia tetracantha* G. S. West Jour. Linn. Soc. Bot. 38: 137, pl. 5, fig. 7, 1907.

This alga is probably only a form of *Pediastrum simplex* (Meyen) Lemmermann.

3. *Tetrastrum apiculatum* (Lemmermann) Brunthaler in Pascher Süßwasserfl. Deut. Oester. u. d. Schweiz 5: 177, fig. 258, 1915; *Staurogenia apiculata* Lemmermann Bot. Centralbl. 76: 151, 1900; Schmidle, Ber. Deut. Bot. Ges. 18: 157, 1900; Snow, Bull. U. S. Fish Comm. 22: 376, pl. 1, fig. V (1-8), 1903; *Crucigenia apiculata* Schmidle, Allg. Bot. Zeitschr. 6: 234, 1900.

This species is very common in the United States, and its habit of forming multiple coenobia (often up to several hundred individuals) places it in the genus *Crucigenia*.

4. *Tetrastrum truncatum* (G. M. Smith) Printz in Engler & Prantl. Die Natur. Pflanzenfamilien 3: 148, 1927; *Crucigenia truncata* G. M. Smith, Phytopl. Inland Lakes Wisc. p. 146, pl. 36, fig. 7-9, 1920.

Designated as *Crucigenia apiculata* Schmidle var. *truncata* (G. M. Smith) Ahlstrom & Tiffany in Tiffany loc. cit. 6: 69, pl. 8, fig. 156, 1934. *C. reniforme* Swirenko (Archiv Russes de Protistol. 5: 85, pl. 4, fig. 16, 1926) is perhaps better considered as a variety of *C. apiculata*.

5. *Tetrastrum multisetum* (Schmidle) Chodat in Süßwasserfl. Deut. Oesterr. u. d. Schweiz 5: 177, 1915; *Staurogenia multiseta* Schmidle, Ber. Deut. Bot. Ges. 18: 157, pl. 6, fig. 12, 1900.

This is only a form of the very variable *Micractinium pusillum* Fresenius.

6. *Tetrastrum rocklandiensis* Griffiths, Jour. Linn. Soc. Bot. 47: 610, text-fig. 6, 1927. Fig. 34.

7. *Tetrastrum tetracanthum* var. *khasianum* Biswas, Hedwigia 74: 19, pl. 3, fig. 3, 1934.

It is difficult to place this alga because of a small and indistinct figure. It appears to be near the "elegans" form described above.

From Griffiths' rather meager description, this species seems to belong more closely to *Micractinium* than to *Tetrastrum*. A definite assignment cannot be made, however, until more is learned about it. It is known from a single specimen in plankton from Rockland Brood, Norfolk, England.

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STUDIES ON THE MORPHOLOGY OF THE ONAGRACEAE.
VIII. *CIRCAEA PACIFICA*

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(Received for publication April 15, 1933)

Our knowledge of the genus *Circaea* has been ably summarized by Gagnepain (1916), who, however, did not have access to a sufficiently large series of herbarium specimens of the Pacific Coast representative, *C. pacifica* Ascherson and Magnus, to feel qualified to make a final disposition of this interesting little species. It may eventually prove to be not much more than a subspecies of *C. alpina* L., but for our present purposes it can be considered to be a distinct species.

This brief study is being presented as a prelude to more extensive investigations upon the genera presumably derived from *Circaea*—namely, *Lopezia*, *Fuchsia*, *Riesenbachia*, etc.

Material. Buds and ovaries were collected in July, 1927, from a small colony of perhaps 25 plants growing in a bog about two miles east of Carlotta, Humboldt County, California. The bog was in a little gully supporting the dense undergrowth typical of clearings in redwood forests; hence the *Circaeas* were in such a situation that they received practically no direct sunlight. When the locality was revisited in 1928, it was found that the colony had completely disappeared. During a period of over seven years, this is the only colony we have come across in our search for *C. pacifica*. Each plant bore only a few flowers; hence not as much material as was desired could be obtained.

We are indebted to Mrs. Charlotte E. Wilder of Carlotta for leading us to the colony, which she originally discovered.

Development of the megagametophyte. Each ovary contains a single anatropous ovule; if a second ovule should arise, it becomes aborted before the megasporocyte originates. The latter develops in the customary manner and is quickly buried deeply in the ovary by the rapid growth of the nucellus and the surmounting tapetal layers of cells (fig. 1). The quartets develop in the fashion typical amongst onagrads, and the micropylar megaspore becomes the functional one (fig. 2). The three non-functional megaspores degenerate rapidly, and all trace of them is soon lost. The functional megaspore becomes globular as it develops (fig. 3, 4), and it is not until organization of the megagametophyte has begun that the embryo sac commences to lengthen. The mature megagametophyte is perfectly regular in organization (fig. 5). The synergids possess shallow indentations, usually lack the characteristic vacuole, and rarely show any evidence of the so-called filiform apparatus.

The egg cell is comparatively small, but possesses an unusually large nucleus in proportion to its size.

Fertilization. The entrance of the pollen tube is markedly irregular. In only two instances was it seen to be strictly porogamous; hence one might

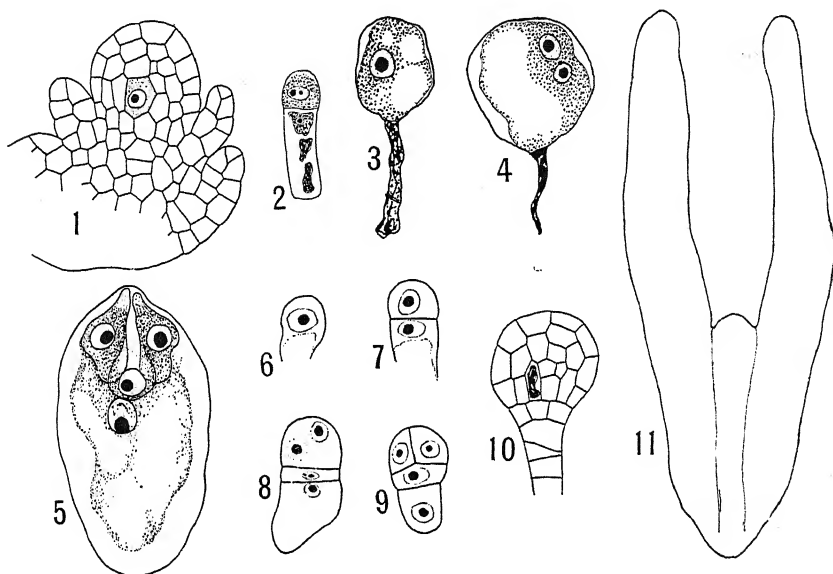


Fig. 1-11. Fig. 1, young ovule, median longitudinal section, shortly after differentiation of megasporocyte. Fig. 2, micropylar megaspore becoming functional. Fig. 3, uninucleate megagametophyte. Fig. 4, binucleate megagametophyte. Fig. 5, mature megagametophyte. Fig. 6, zygote. Fig. 7, two-celled embryo. Fig. 8, four-celled embryo. Fig. 9, quadrant stage. Fig. 10, later stage showing diseased cell in center. Fig. 11, nearly mature embryo. All $\times 475$.

conclude that the microgametophyte enters the ovular locus in a hit-or-miss fashion. It usually comes into contact with the thin outer integument, which completely encloses the ovule, penetrates both integuments, travels up to the apex of the nucellus between the latter and the inner integument, and then traverses the tapetal layers of cells to the embryo sac. Fertilization itself, as observed in two embryo sacs only, appears to be normal.

Embryogenesis. As may be seen if the various stages (fig. 6-11) are compared with those of *Godetia amoena*, which we consider to be the "standard" for the family (Johansen, 1930), the embryology of *C. pacifica* conforms to type. In the intermediate stages of growth it is difficult to identify the tissues derived from the hypophysis cell—a not uncommon occurrence in other species as well. In a few embryos isolated diseased cells (caused by bacteria?) were noticed, but the embryo is apparently able to survive such attacks. No irregularities or abnormal structures were encountered, although such might have been found if a longer series of preparations had been avail-

able for study. When the seed is ripe, the embryo fills the entire ovule, leaving only the nucellar epidermis intact within the inner integument; the ovary is indehiscent. The cotyledons (fig. 11) are very thin and alate.

Endosperm. The coenocytic endosperm is frequently copious in many embryo sacs before the growing embryo begins to fill the latter. The nucleoli attain an exceptionally large size. It was possible to count the chromosomes in several well fixed mitotic figures; the total number is that previously reported as the diploid number for the species ($2n=22$). The endosperm is finally completely absorbed by the embryo.

SUMMARY

The ovary of *Circaea pacifica* Asch. & Mag. is uniovular. Megasporogenesis and embryogenesis both follow the developmental schemes considered to be typical for the Onagraceae as a whole. No irregularities of any nature were observed.

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AN ANATOMICAL STUDY OF TRAUMATIC AND OTHER ABNORMAL TISSUES IN *CARNEGIEA GIGANTEA*

ANSEL F. HEMENWAY

(Received for publication April 10, 1933)

Traumatic tissues in *Carnegiea gigantea* are formed apparently in response to acute wounds made by the plant's natural enemies, birds and insects, and to general surface irritation or weathering. The two types are the same in color, a brownish-gray, and in general texture, though the former is more "horny" and the latter more bark-like and scaly in appearance. The first purpose of this investigation was to make an anatomical analysis and comparison of the two types. Later, other abnormal vascular structures were studied.

There is very little literature on the anatomy of the Cactaceae, and nothing was found concerning traumatic tissue in *Carnegiea gigantea*, although the plant, according to MacDougal (1905), was first mentioned as early as 1604.

Ganong (1895) merely makes the general statement that "perfect cork" occurs in cacti.

Schleiden (1839), whose detailed anatomical study of a number of genera of this family is unique, describes and illustrates cork formation in *Opuntia* and *Echinocactus*, remarking that this cork is made up of lamellae due to the alternation of layers of thick- and thin-walled cells. He agrees with those whom he calls "the older anatomists" that the bark (by which he declares they usually mean "cork") is an epidermis, and goes on to say that the tissue around wounds and the "cork" (referring to the bark-like type) are identical. His final conclusion is that all cork is a form of traumatic tissue.

Scott (1932), in her study of *Fouquieria*, which is somewhat close to the Cactaceae, states that the cork cambium produces unthickened cork cells and thick-walled "fibrous cork cells." In reference to the "fibrous cork," she says, "As is seen from micro-chemical tests only the middle lamella is suberized, and the wall thickening is due to a heavy lining of cellulose."

The extreme hardness of this "fibrous cork" seemed to indicate that lignin must be present, and made it seem necessary to check her last statement carefully. It was found that in a phloroglucinol-hydrochloric-acid test, if left for at least twenty minutes, the "heavy lining of cellulose" becomes a pale violet-red, giving a reaction about one-third as intense as that of ordinary lignified tissue, and showing the presence of some lignin.

[The JOURNAL for October (21: 427-511) was issued October 1, 1934.]

AMERICAN JOURNAL OF BOTANY, VOL. 21, NO. 9, NOVEMBER, 1934

Solereder (1908), though he does not mention the Cactaceae, declares that "in certain plants . . . the cork contains unsuberized cells having walls, which consist of cellulose or are even more or less lignified (phelloid-cells) . . .," and farther on, that "layers of phelloid-cells have been recorded in the following Orders: Hypericineae, Burseraceae, . . . Rosaceae, Combretaceae, Myrtaceae, Melastomaceae, Lythrariceae, Onagrariceae, Caprifoliaceae, Penaeaceae."

In making the study which follows, free-hand sections of living material were used for the usual microchemical tests.

Cubes one-third of an inch in diameter were cut from the inner portion of tissue around wounds and "bark," so as to include cambium and a little adjacent fundamental tissue. Material was killed in a solution of corrosive sublimate, acetic acid, and picric acid. When it was found that the ordinary hydrofluoric method did not give satisfactory results, the material was placed in full-strength diaphanol for two weeks. It was then imbedded in paraffin or celloidin and 10-12-micron sections were cut on a sliding microtome. When crystals of calcium oxylate caused the fundamental tissue to tear, they were removed in a 4 per cent hydrochloric acid solution.

Practically every mature specimen of *Carnegiea gigantea* is a victim of the excavations of birds, especially the Gila woodpecker, and to a less serious extent, of the invasions of several species of insects. In order to investigate the formation and development of traumatic tissue in response to such an attack, in September, 1929, an artificial wound was made in the branch of a large old specimen. A mucilaginous secretion first dried around the wound, the outer layer of cells dried up, and immediately thereafter the wound cambium began to develop a layer of traumatic tissue. In September, 1930, this tissue was examined microscopically and was found to consist, in cross section, of four layers.

First was a layer of about forty thick-walled, brick-shaped cells in compact radial rows, which were, in general appearance, cork-like, but were actually lignified; second, a layer of four or five thinner-walled true cork cells, more or less crushed; third, a layer of about twenty thicker-walled cells like the first; fourth, a layer of one or two cambium cells.

The thick-walled cells gave a striking lignin test with phloroglucinol. When first formed, they sometimes gave a slight suberin test at the corners of the primary wall, but never in the secondary wall. The thin-walled cells gave the suberin test with Soudan III and also with Scarlet Red.

The cambium consisted of thin-walled, flat cells, quite narrow in the radial plane, but three to four times broader in the vertical and tangential planes.

The success of the cactus in protecting its tender tissues in this way from the effects of acute internal wounds is naturally limited to injuries which are somewhat localized. In the case of general serious injury, such as, for instance, the result of a bolt of lightning, the cortex and pith tissues ferment so rapidly, especially in hot weather, when the alcoholic stage may be reached

within two or three days, that the plant is apparently unable to develop protective tissue rapidly enough to save itself.

Weathering of the surface gives rise to the second type of traumatic tissue mentioned in the introduction. *Carnegiea gigantea* may live, it has been estimated, from one to perhaps two or three hundred years. At what we may then assume to be about fifty years of age, typical specimens develop near the ground level a protective covering of bark-like, indurated tissue which gradually spreads upwards, until very old plants may be sheathed for a distance of six or more feet from the ground (fig. 3).

It is interesting to note that this tissue usually appears first on the side most frequently exposed to the prevailing local sand storms. Sometimes it begins on the spiny ridges, but oftener in the more openly exposed furrows. Where a plant is not solitary, but stands in a group of its own kind or among trees and shrubs, the protection afforded by its neighbors is clearly shown in the belated and less extensive development of this type of tissue. A similar but usually more scaly type of "bark" appears on any roots that chance to be exposed.

This type of traumatic tissue was found to have a microscopical structure similar to that which is formed around wounds. It is composed of thick-walled, regularly placed, lignified cells in layers three to six times thicker than the alternating suberized layers.¹ The latter are not independently concentric, but on the contrary often branch, much like the cork layers in the bark of the deciduous oak (fig. 2).

The lignified cells have many simple pits which are cone-shaped, with the apices at the primary wall, and are therefore quite different from the slender pits characteristic of stone cells.

The thin cork cells eventually break, thus causing the "bark" or other wound tissue to come off in thin plates.²

Both of the foregoing types of traumatic tissue are exceedingly tough and resistant to decay. After the fundamental tissue has entirely disintegrated, it is possible to pick up these indurated structures intact (fig. 1). In the case of those made after bird excavations, specimens may range up to two feet in length. Small, round, pearl-like, or baroque pieces were probably caused by insect injuries. In some of the more perfect examples the invader's point of entry may be entirely obscured.³

For purposes of comparison, the traumatic tissues of several *Opuntias*

¹ In Schleiden's (1839) drawing (pl. IX, fig. 5) "c" is used to label the lignified layers which he calls "thick-walled cork," while "d" includes the two layers of "thin-walled cork" (p. 44). The inner "d" layer is actually the cork cambium, which he seems not to have recognized, and of which he admits (p. 18) he cannot explain the origin. Failure to recognize the cambium led him into the error of classifying the cork as "the only true endogenous structure of the plant" (p. 19).

² Schleiden (1839) recognized this lamination in *Opuntia monacantha* (p. 19).

³ Described and illustrated by Isabel Hemenway in "Cactus Pearls," *Nature Magazine*, November, 1932.

and of *Echinocactus wislizeni* were examined and found to be similar in structure to those of *Carnegiea gigantea*. Moreover, Miss Alice McLaughlin, who is working on fungus diseases of the Cactaceae, reports that the tissue formed by the cork cambium of *Cereus schottii* is also almost identical with that of *Carnegiea gigantea*.

In preliminary conclusion, it may then be said that the cork cambium of *Carnegiea gigantea*, like that reported for *Fouquieria splendens* (Scott, 1932), produces two types of cells, thick-walled and thin-walled, in alternate layers. Both in the tissue around wounds and in the "bark" of *Carnegiea gigantea*, these thick-walled cells are strongly lignified, while the thin-walled cells are suberized. The former occur in layers three to six times thicker than those of the latter.

On further investigation, another peculiar and possibly traumatic formation was found to occur in *Carnegiea gigantea*.

At the base of more than half the branches on old large plants, root-like, vascular strands develop, usually below, but occasionally above or at the side of the branch. These strands depart from the vascular cylinder and grow down or around in the cortex of the main axis; here they may turn inward and join the vascular cylinder of the main axis (fig. 4, 5), or occasionally they may grow down the cortex for two or more feet, anastomosing somewhat in the manner of the vascular structure of an *Opuntia*. Eventually some of the smaller branches turn in and join the vascular system of the main stem. In other cases, vascular strands seem to reverse this process i.e., they emerge from the main vascular cylinder, growing out through the cortex and joining with the vascular structure of the branch.

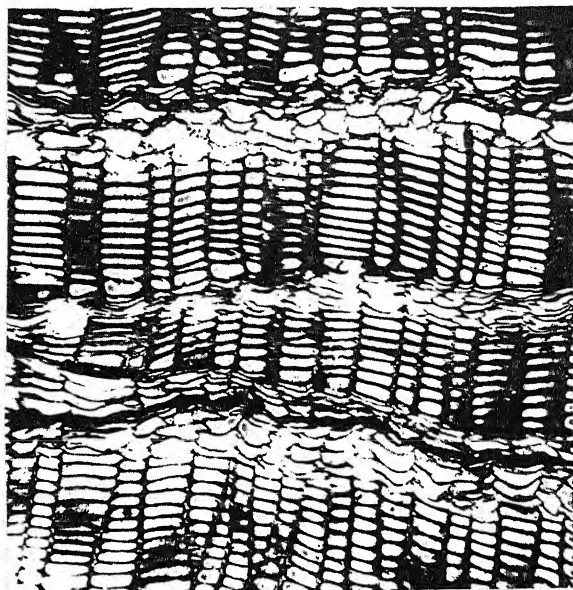
These vascular strands may be flat, with phloem on the outside of the xylem, or nearly round, with phloem practically surrounding the xylem.

In the case of young branches, after vascular strands are cut by a woodpecker, the new layers of vascular tissue formed may be extended in such a way as to be reunited around the wound. An artificial reproduction of this condition is being attempted for purposes of more satisfactory study.

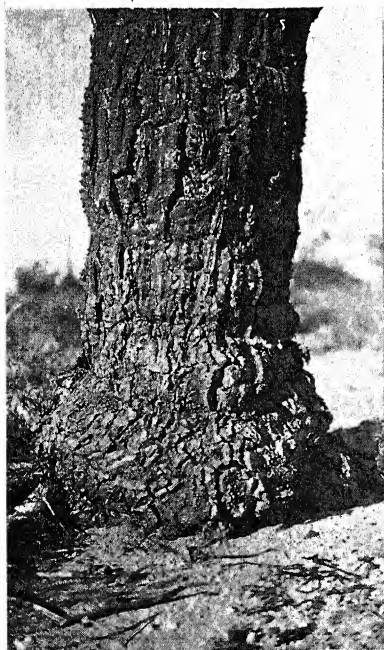
Fig. 1-5. Fig. 1. Wound tissue formed around the cavity made by a woodpecker. Entrance was at upper right point. Fig. 2. Cross section of "bark" of *Carnegiea gigantea*, showing layers of thick-walled, rectangular lignified cells alternating with thinner layers of thin-walled, irregular suberized cells. \times about 450. Fig. 3. Base of a mature *Carnegiea gigantea*, showing scaly, bark-like covering. Fig. 4. Part of woody skeleton of *Carnegiea gigantea*, showing a root-like strand emerging from underside of a branch near its base, extending downward, then bending abruptly back and joining the woody portion of the main stem. Just above the base of the branch is a flattened vascular strand growing out horizontally from the main stem, anastomosing to some extent, and then uniting with the vascular system of the branch. Fig. 5. Similar to fig. 4, showing at top fusion of anastomosed strands and at side root-like strands emerging from main stem and reuniting above with main stem. On the opposite side of the skeleton, and not shown, is hole made by a woodpecker which probably caused all these abnormalities.



1



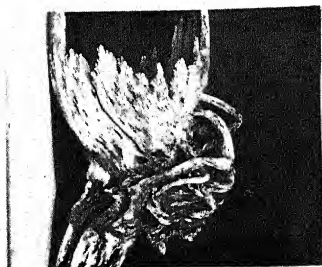
2



3



4



5

Occasionally the vascular skeleton of a specimen, instead of forming the typical elongated, nearly parallel ribs, will anastomose, apparently reverting to the more primitive cylindro-opuntia type. Again, for a distance of from twelve to fourteen inches, the network of such a vascular system may be so close as to form an almost solid-walled cylinder (fig. 5).

Isolated vascular strands consisting of one or more vessels and some phloem may occur in either the pith or cortex of young, thrifty plants, apparently formed in healthy, uninjured tissue. Where these occur at the base of branches, it may be possible that under certain conditions they are enlarged into the peculiar structures just described. Since branches of this cactus are practically rigid, it is reasonable to assume that they are subjected to heavy strain at the base, especially in times of storm. Perhaps this may account for these abnormal developments.

In *Carnegiea gigantea*, roots also often fuse, showing the readiness with which vascular strands of this plant grow together. In such cases the xylem portions may unite as firmly and smoothly as two adjacent ribs of the main stem.

All these widely differing types of structure, in testifying to the plastic development of the Cactaceae, may perhaps help to explain their success in surviving extremely unfavorable environmental conditions.

SUMMARY

Carnegiea gigantea protects itself from external and internal injuries by developing cork-like tissue composed of thick-walled, heavily lignified cells alternating with thinner layers of thin-walled, suberized cells. Near the base of large branches, root-like strands of vascular tissue may pass obliquely down through the cortex to join the main vascular system, or they may go from the main cylinder to the branch. These apparently serve as props. The usual parallel rib structure may anastomose so as to resemble the vascular system of an *Opuntia*, or, when severely injured, may form a solid woody cylinder.

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FERTILIZATION IN *ASCODESMIS NIGRICANS* VAN TIEGH.

DEANE B. SWINGLE

(Received for publication April 17, 1933)

Among the cytological problems found in the fungi, few present greater interest, or greater perplexity, than does that of the sexuality of the Ascomycetes. Unfortunately certain aspects of this problem have become so involved in controversy that a considerable amount of new and of confirmatory work will be required to establish the facts.

HISTORICAL

When Dangeard (1894) observed nuclear fusion in the ascus of *Peziza vesiculosa* Bull. and several other fungi, he made an important discovery, but unfortunately he blinded himself to the presence and significance of the antheridia and oogonia described by earlier workers and interpreted the ascus as an egg cell and the nuclear fusion that he observed there as a sexual process.

The next year Harper (1895), reporting his studies of *Sphaerotheca humuli*, confirmed Dangeard's observation of nuclear fusion in the ascus but showed that it is preceded by a regular fertilization process in which the nucleus from an antheridium is discharged into an oogonium where nuclear fusion takes place, followed by the outgrowth of an ascogenous hypha, the penultimate cell of which becomes an ascus containing two nuclei which fuse. This, then, constitutes the second fusion in the life cycle, and Harper's interpretation differs fundamentally from that of Dangeard, for Harper considers the nuclear fusion in the oogonium rather than that in the ascus the true conjugation process.

This work, and later publications by these and other investigators, have stimulated wide interest among cytologists. Working on a wide range of Ascomycetes and Ascolichenes, some have contributed thorough-going cytological work of merit, some have published work essentially morphological, with an incidental effort at cytological study, while still others have expressed opinions that were not based on observations of their own.

For brevity and for convenience of discussion the writer has assembled in tabular form the reports of the chief contributors to our knowledge of fertilization in the Ascomycetes. No pretense is made of completeness, for papers on this subject range all the way from the most casual and imperfect observations to cytological research of a high order, and the line of demarcation is not sharp. Only those papers are included in which an opinion is

offered, supported by first-hand evidence, on the question of whether or not a nuclear fusion takes place prior to that in the ascus. The present work on *Ascodesmis nigricans* Van Tiegh. is included for purposes of comparison.

This table shows the diversity of opinion that exists with regard to nuclear fusion in the ascogonium. This may be due in part to actual differences occurring among the various species and varieties of Ascomycetes but doubtless is attributable also to faulty observations. It will be seen that the greatest confusion exists with regard to *Pyronema*.

In the family Pyronemaceae the only genera that have been studied in cytological detail are *Pyronema* and *Ascodesmis*. Harper (1900) gave the first account of a cytological study of *P. confluens* based on modern technique. He traced the formation and development of the sex organs, and the entire course of fertilization, spore formation, and the development of the ascocarp. According to this investigator both antheridium and oogonium are multinucleate, and the nuclei of the two are not greatly different in size. A unicellular, multinucleate trichogyne (conjugation tube) is developed at the top of the oogonium with a septum at its base. The trichogyne and antheridium become intertwined in their development, and the beak of the former presses tightly against the latter. Harper traced consecutively the disintegration of the trichogyne nuclei, the formation of an opening between its beak and the antheridium, the passage of the antheridial nuclei through the trichogyne into the oogonium, and their fusion there with the female nuclei.

The evidence of nuclear fusion in the oogonium merits especial consideration, as it is the portion of the work that has come most into controversy. After the nuclei from the antheridium had entered the oogonium, there was observed to be considerable pairing. Harper's descriptions and figures show some nuclei lying apart, some in contact with each other, and some with openings through adjacent nuclear membranes. After opportunity for fusion had been offered, some of the nuclei were large and some were small, although all were approximately of the same size before. In his figures some of the larger nuclei show two nucleoli. He was not able to make counts showing a diminution in the total number of nuclei, as the number is very large and during fertilization they are densely aggregated in the center of the oogonium. They leave this mass to enter the complicated system of ascogenous hyphae, where only part of them can be followed in sections.

Each ascogenous hypha, which by branching is destined to form several asci, receives a considerable number of nuclei. These showed no marked tendency to occur in pairs after entering the hyphae, although in his figure 20, two nuclei in contact can sometimes be found. A few small ones which Harper regarded as supernumerary oogonial, or possibly antheridial, nuclei remained in the ascogonium or migrated into the bases of the ascogenous hyphae. They appeared to disintegrate without taking part in ascus formation.

TABLE I. *Summary of the work on conjugation in the Ascomycetes*

Name of fungus	Origin of nuclear pair	No. of nuclei in oogonium*	No. of asci per ascogonium	Trichogyne present	Origin of ascus†	Nuclear fusion in ascogonium		Authority	Reference	Date
						Occurrence	Evidence ‡			
<i>Amauroascus verrucosus</i> (Plectascales)	Both oogonial	Several	Several	No	—	No	None observed	Dangeard, P. A.	Le Botaniste 10: 1	1907
<i>Ascobolus citrinus</i> (Pezizales)	Both oogonial	Several	Several	No?	Penultimate cell	No	None observed, only pairing	Schweizer, G.	Zeit. Bot. 15: 529	1923
<i>Ascobolus furfuraceus</i> (Pezizales)	Both oogonial	Several	Several	No	Penultimate cell	Yes	Observed fusion stages	Welsford, E. J.	New Phytol. 6: 156	1907
"	—	—	—	—	—	Yes	Brachymyiosis	Fraser, H. C. I., and Brooks, W. E. St. J.	Ann. Bot. 23: 537	1909
<i>Ascobolus immersus</i> (Pezizales)	Both oogonial	Several	Several?	—	Penultimate cell	No	None observed, only pairing	Ramlow, G.	Myc. Centralb. 5: 177	1915
<i>Ascobolus strobilinus</i> (Pezizales)	Antheridium and oogonium	Several	Several	Yes	—	No	None observed, only pairing	Schweizer, G.	Planta 12: 588	1931
<i>Ascodesmis nigricans</i> (Pezizales)	Antheridium and oogonium	Several	Several	Yes	Penultimate cell	Yes	Observed fusion stages	Claussen, P.	Bot. Zeit. 63: 1	1905
"	—	—	—	—	—	No	Inference from Pyronema	"	Zeit. Bot. 4: 1	1912
"	Antheridium and oogonium	Several	Several	Yes	Penultimate cell	Yes	Observed fusion stages	Swingle, D. B.	Amer. Jour. Bot. 21: 519	1934
<i>Ascobolus magnificus</i> (Pezizales)	Antheridium and oogonium	"	"	"	Penultimate cell	Yes	Observed fusion stages. Brachymyiosis	Gwynne-Vaughan, H. C. I., Williamson, H. S.	Ann. Bot. 46: 653	1932
<i>Ascophanus carneus</i> (Pezizales)	Both oogonial	Several	Several	Yes?	Penultimate cell	Yes	Observed fusion stages	Cutting, E. M.	Ann. Bot. 23: 399	1909
"	Both oogonial	Several	Several?	—	Penultimate cell	No	None observed, only pairing	Ramlow, G.	Myc. Centralb. 5: 177	1915
<i>Ascophanus ochraceus</i> (Pezizales)	Both oogonial	Several	Several	—	Penultimate cell	No	None observed	Dangeard, P. A.	Le Botaniste 10: 1	1907
<i>Ceratostomella fimbriata</i> (Sphaeriales)	Antheridium and oogonium	One	Several	Yes	Terminal cell	Yes	Observed fusion stages. No. of nuclei	Elliott, J. A.	Phytopath. 15: 417	1925
"	Both oogonial	One	Several	Yes?	Division of oogonial cell	No	None observed	Andrus, C. F., Harter, L. L.	Jour. Agr. Res. 46: 1059	1933
<i>Cryptomyces Pteridis</i> (Phacidiales)	Two oogonia	One	Several	No?	—	No	None observed. Persistent pairing. No brachymyiosis	Killian, K.	Zeit. Bot. 10: 49	1918
<i>Dipodascus albidus</i> (Plectascales)	Antheridium and oogonium	Several	One	No	Directly from ascogonium	Yes§	Presence of large nucleus	Juel, H. O.	Flora 91: 47	1902

TABLE I—Continued.

Name of fungus	Origin of nuclear pair	No. of nuclei in oogonium*	No. of asci per ascogonium	Trichogyne present	Origin of ascus†	Nuclear fusion in ascogonium		Authority	Reference	Date
						Occurrence	Evidence ‡			
"	Antheridium and oogonium	Several	One	No	Directly from ascogonium	Yes§	Observed fusion stages	Dangeard, P. A.	Le Botaniste 10: 1	1907
<i>Endomyces Magnusi</i> (Plectascales)	Antheridium and oogonium	One	One	No	Directly from ascogonium	Yes§	Observed fusion stages	Guilliermond, A.	Rev. Gen. Bot. 21: 353	1909
<i>Eremascus fertilis</i> (Plectascales)	Antheridium and oogonium	One	One	No	Directly from ascogonium	Yes§	One nucleus from two	Stoppel, Rose	Flora 97: 332	1907
<i>Eremascus fertilis</i> (Plectascales)	Equal gametes	One	One	No	Directly from gamete pair	Yes§	Observed fusion stages	Guilliermond, A.	Rev. Gen. Bot. 21: 353	1909
<i>Erysiphe Polygoni</i> (Erysiphales)	Antheridium and oogonium	One	Several	No	Intercalary cells	Yes	Observed fusion stages. Nuclear numbers	Harper, R. A.	Jahrb. Wiss. Bot. 29: 655	1896
"	Both oogonial	One	Several	No	—	No	None observed	Dangeard, P. A.	Le Botaniste 10: 1	1907
<i>Helvella crispa</i> (Helvellales)	Apogamous	—	—	No	Penultimate cell	Yes	Observed fusion stages. Brachymeiosis	Carruthers, D.	Ann. Bot. 25: 243	1911
<i>Humaria granulata</i> (Pezizales)	Both oogonial	Several	Several	No?	Usually penultimate cells	Yes	Observed fusion stages	Blackman, V. H., Fraser, H. C. I.	Proc. Roy. Soc. Lond. B 77: 354	1906
"	—	—	—	—	—	Yes	Brachymeiosis	Fraser, H. C. I., Brooks, W. E. St. J.	Ann. Bot. 23: 537	1909
<i>Humaria rutilans</i> (Pezizales)	Apogamous	—	—	No	Penultimate cell	Yes	Observed fusion stages. Brachymeiosis	Fraser, H. C. I.	Ann. Bot. 22: 35	1908
<i>Laboulbenia chaetophora</i> (Laboulbeniales)	Both oogonial	One	Several	Yes	Aseogenic cell	No	None observed. No brachymeiosis	Faull, J. H.	Ann. Bot. 26: 325	1912
<i>Lachnea scutellata</i> (Pezizales)	Both oogonial	Several	Several	Not stated	Penultimate cell	No	None observed. No brachymeiosis	Brown, W. H.	Bot. Gaz. 52: 275	1911
"	Not determined	"	"	"	Not stated	Yes	Brachymeiosis	Gwynne-Vaughan, H. C. I., Williamson, H. S.	Ann. Bot. 47: 375	1933
<i>Lachnea stercorea</i> (Pezizales)	Both oogonial	Several	Several	Yes	Penultimate cell	Yes	Observed fusion stages	Fraser, H. C. I.	Ann. Bot. 21: 349	1907
"	—	—	—	—	—	Yes	Brachymeiosis	Fraser, H. C. I., Brooks, W. E. St. J.	Ann. Bot. 23: 537	1909
<i>Monascus purpureus</i> (Plectascales)	Antheridium and oogonium	Several	Several	Yes	Penultimate cell	No	None observed, only pairing	Schikorra, W.	Zeit. Bot. 1: 379	1909
<i>Morchella deliciosa</i> (Helvellales)								Wakayama, K.	Cytologia 2: 27	1930

TABLE I.—Continued.

Name of fungus	Origin of nuclear pair	No. of nuclei in oogonium*	No. of asci per ascogonium	Trichogyne present	Origin of ascus†	Nuclear fusion in ascogonium		Authority	Reference	Date
						Occurrence	Evidence ‡			
<i>Mycosphaerella cerasella</i> (Sphaeriales)	Both oogonial?	Several	Several	Yes	Terminal cell	No	None observed, only pairing	Jenkins, W. A.	Phytopath. 20: 320	1930
<i>Mycosphaerella personata</i> (Sphaeriales)	? and oogonium	One	Several	Yes	Penultimate cell	No	None observed, only pairing	Higgins, B. B.	Amer. Jour. Bot. 16: 287	1929
<i>Peltigera</i> four species (Lichens)	Both oogonial	Several	Several	No	Terminal cell	No	None observed. No brachymeiosis	Moreau, M. and Mme. F.	Compt. Rend. 160: 526	1915
<i>Peziza vesiculosa</i> (Pezizales)	Apogamous?	—	—	No	Penultimate cell	Yes	Brachymeiosis	Fraser, H. C. I., Welsford, E. J.	Ann. Bot. 22: 465	1908
<i>Phyllactinea corylea</i> (Erysiphales)	Antheridium and oogonium	One	Several	No	Terminal or sub-terminal cell	Yes	Observed fusion stages. Nuclear numbers	Harper, R. A.	Publ. Carnegie Inst. Washington 37	1905
<i>Polystigma rubrum</i> (Hypocreales)	Apogamous	—	—	No	Penultimate cell	Yes	Nuclear pairing and size	Blackman, V. H., Welsford, E. J.	Ann. Bot. 26: 761	1912
"	Both oogonial	One	—	Yes	—	No	None observed, only pairing	Nienburg, W.	Zeit. Bot. 6: 369	1914
<i>Pyronema confuens</i> (Pezizales)	Antheridium and oogonium	Several	Several	Yes	Penultimate cell	Yes	Observed fusion stages	Harper, R. A.	Ann. Bot. 14: 321	1900
"	Both oogonial	Several	Several	Yes?	Penultimate cell	No	None observed	Dangeard, P. A.	Le Botaniste 10: 1	1907
"	Antheridium and oogonium	Several	Several	Yes	Penultimate cell	No	None observed, only pairing	Claussen, P.	Zeit. Bot. 4: 1	1912
"	Antheridium and oogonium	Several	Several	Yes	Penultimate cell	Yes	Observed fusion stages. Brachymeiosis	Gwynne-Vaughan, H. C. I., Williamson, H. S.	Ann. Bot. 45: 355	1931
"	Both oogonial	Several	Several	Yes	Penultimate cell	No	None observed. No brachymeiosis	Moreau, M. and Mme. F.	Rev. Gen. de Bot. 43: 465	1931
<i>Pyronema confuens</i> var. <i>igneum</i>	Both oogonial	Several	Several	Yes	Penultimate cell	No	None observed. No pairing	Brown, W. H.	Amer. Jour. Bot. 2: 289	1915
<i>Pyronema domesticum</i>	Antheridium and oogonium	Several	Several	Yes	Penultimate cell	Yes	Observed fusion stages. Brachymeiosis	Tandy, G.	Ann. Bot. 41: 321	1927
<i>Rhizinia undulata</i> (Helvellales)	Both oogonial	Several	Several	Yes?	Penultimate cell	No	None observed, only pairing	Fitzpatrick, H. M.	Bot. Gaz. 65: 201	1918
<i>Sphaerotheca humuli</i> (Erysiphales)	Antheridium and oogonium	One	One	No	Penultimate cell	Yes	Observed fusion stages. Nuclear numbers	Harper, R. A.	Ber. Deut. Bot. Ges. 13: 475	1895
<i>Sphaerotheca humuli</i> (Erysiphales)	Antheridium and oogonium	One	One	No	Penultimate cell	Yes	Observed fusion stages. Nuclear numbers	Blackman, V. H., Fraser, H. C. I.	Ann. Bot. 19: 567	1905

TABLE I—*Continued.*

Name of fungus	Origin of nuclear pair	No. of nuclei in oogonium*	No. of asei per ascogonium	Trichogyne present	Origin of ascus†	Nuclear fusion in ascogonium		Authority	Reference	Date
						Occurrence	Evidence ‡			
"	Both oogonial	One	One	No	Penultimate cell	No	None observed	Dangeard, P. A.	Le Botaniste 5: 245	1897
"	Both oogonial	One?	One	—	Penultimate cell	No	None observed	Winge, O.	Bull. Soc. Myc. France 27: 211	1911
"	Both oogonial	One	One	No	Penultimate cell	No	None observed	Moreau, M. and Mme. F.	Rev. Gen. Bot. 42: 65	1930
<i>Taphrina epiphylla</i> (Exoascales)	Two conidia	—	—	No	—	No	Nuclear numbers	Wieben, Magdalene	Forsch. Geb. Pflanzenkr. 3: 139	1927
<i>Taphrina Klebahnii</i>	Two conidia	—	—	No	—	No	Nuclear number	Wieben, Magdalene	Forsch. Geb. Pflanzenkr. 3: 139	1927
<i>Thelebolus stereoreus</i> (Pezizales)	Both oogonial	Two	One	No?	Directly from ascogonium	Yes§	One nucleus from two	Ramlow, G.	Bot. Zeit. 64: 85	1906
<i>Venturia inaequalis</i> (Sphaeriales)	Antheridium and oogonium	Several	Several	Yes	Penultimate cell	No	None observed	Killian, K.	Zeit. Bot. 9: 353	1917
<i>Venturia inaequalis</i> (Sphaeriales)	Antheridium and oogonium	Several	Several	Yes	Directly from ascogonium	No§	None observed, only pairing	Frey, C. N.	Trans. Wis. Acad. Sci. 21: 303	1924

* All indefinite numbers of oogonial nuclei are recorded as "several."

† Penultimate cell refers to the ascogenous hypha.

‡ Extended arguments for or against nuclear fusion in the ascogonium cannot be included in this table for want of space.

§ In these species but one nuclear fusion in the life cycle is recorded. Whether this should be interpreted as ascogonial fusion or as ascus fusion is debatable. See discussion, page 536.

He observed no mitosis in the ascogenous hyphae until the time of ascus formation, when two nuclei divided simultaneously to form four, of which two, not sisters, remained in the penultimate cell, which developed into the ascus, while one of the others came to occupy the tip cell and the other the basal cell.

In the ascus nuclear fusion, followed by nuclear division and the formation of eight spores, was observed to follow the usual course described for the Ascomycetes.

Harper recognized the desirability of determining the chromosome numbers at different stages of development in *Pyronema confluens*. He was unable to accomplish this with certainty, however, or to determine the point at which chromosome reduction takes place.

Dangeard (1907) also investigated *Pyronema*, still using whole mounts, however, rather than paraffin sections. On most points of morphology he agreed with Harper, but he admitted no passage of nuclei from the antheridium through the trichogyne into the oogonium.

Shortly after the publication of Harper's work on *Pyronema confluens*, Claussen began a cytological study of the same fungus and has published three papers (1906, 1907, 1912) reporting his observations. He confirmed Harper's findings in most particulars, the only major point of deviation being with regard to the fusion of the nuclei in the oogonium and the consequent significance of fusion in the ascus. Only this aspect of Claussen's work need therefore be reviewed here. He found a very definite pairing of nuclei in the ascogonium and maintained that these pairs do not fuse before entering the ascus. Instead, they migrate in pairs through the ascogenous hyphae, where they divide simultaneously. Finally, after the ascus has been formed from the penultimate cell, it contains two nuclei, one of which, presumably, is a direct descendent from an antheridial nucleus, and the other from an oogonial nucleus. He noted that in some cases, after the ascus has formed, the tip cell and the basal cell (antepenultimate) unite to form another ascus, a phenomenon that has since been reported as occurring widely among the Ascomycetes.

In determining chromosome numbers at different stages of development, Claussen was scarcely more successful than Harper. In the telophase of nuclear division in the tip of the ascogenous hypha he was fairly certain there were twelve chromosomes. In diakinesis of the primary ascus nucleus he reported about twelve pairs. In succeeding divisions the number remained nearly enough the same to preclude, in his judgment, chromosome reduction.

Brown (1915) gave the results of a cytological investigation of a form of *P. confluens* which he designated as var. *inigneum* Brown. It differs chiefly from the typical species in the disinclination of its antheridia and oogonia to unite. Brown reported that in the absence of fertilization the nuclei of the trichogyne disintegrate, and, considerably later, those of the antheridium also. Ascogenous hyphae grew from the ascogonia, and some of the ascogonial nuclei entered them. No tendency to nuclear pairing or fusion was found previous to ascus formation. Nuclear fusion in the ascus took place, presumably, between nuclei derived from those in the oogonium only. The chromosome number was apparently five "through all the divisions of the life history of the plant." No illustrations were submitted with this paper.

Tandy (1927) studied *P. domesticum* Sow., which he states is closely allied to *P. confluens*. He described the discharge of antheridial nuclei into the oogonium, and a pairing and fusion of nuclei there. He gave special attention to chromosome numbers, finding the haploid number, as shown in germinating spores, to be seven. In the nuclear division at the end of the ascogenous hypha, where the ascus pair is being formed, the number was haploid in some cases and diploid in others. Correspondingly in the division of the definitive ascus nucleus the number was sometimes diploid and sometimes tetraploid. From these observations Tandy reasoned that some of the oogonial and antheridial nuclei united before reaching the ascus and became diploid, while others did not unite and remained haploid. More or less nuclear

pairing was noted in the vegetative mycelium and in antheridia and oogonia before fertilization as well as in the ascogenous hyphae.

Further study of the nuclear behavior in *Pyronema confluens* has been made by Moreau and Moreau (1930). They observed union of antheridium with trichogyne and even an opening through the adjacent walls. They deny, however, that there is any passage of antheridial nuclei through this opening, or that there is a nuclear fusion or even a special tendency to pairing of nuclei in the ascogonium or in the lower part of the ascogenous hyphae as reported by Claussen. With respect to other details they are essentially in harmony with previous workers.

A very thorough study of the cytology of *Pyronema confluens*, made in the light of the controversies of previous investigators, was published by Gwynne-Vaughan and Williamson (1931). They described the formation of an opening between antheridium and trichogyne, the passage of male nuclei, and the subsequent closing of this pore. Numerous counts were made of the numbers of nuclei. It was found approximately to double with the entrance of male nuclei and to reduce to approximately the original number after time for fusion in the oogonium had elapsed. Stages in nuclear fusion were described and figured.

Nuclear division was studied in the ascogenous hyphae, in the croziers at their tips, and in the asci. The chromosome numbers were definitely established as follows: six in the germinating spores and the somatic cells of the gametophyte, twelve in the nuclei of the ascogenous hyphae (sporophyte), twenty-four (twelve pairs of gemini) in the first division of the definitive ascus nucleus, reducing to twelve, and twelve in the second ascus division, reducing to six, which continued through the third division. Serious doubt was cast on Claussen's interpretation of the nuclear pairs found in ascogenous hyphae.

More conclusively than any previous investigators Gwynne-Vaughan and Williamson have established two successive nuclear fusions in *Pyronema*.

However, Moreau and Moreau (1931) disagree in part with Gwynne-Vaughan and Williamson as to the chromosome numbers in different stages of the life history of *Pyronema*. They report twelve chromosomes in the nuclei of the ascogenous hyphae and twelve gemini (equivalent to twenty-four chromosomes) in the first division of the definitive ascus nucleus, with reduction to twelve chromosomes in that nuclear division. This number persists, according to these authors, through the second and third divisions of the ascus nuclei which would make the haploid number twelve.

Ascodesmis has received much less study than has *Pyronema*. The genus was established by Van Tieghem (1876), with two species, *A. nigricans* and *A. aurea*. Some half-dozen species are now recognized.

Zukal (1886) grew the fungus on bits of dog dung in moist chambers on microscope slides and studied its morphology. He evidently saw the beginnings of sex organs, but did not recognize them as such and concluded that there was no sexuality.

Dangeard (1903a, 1903b) described the origin and structure of the multiple coils of antheridia and oogonia but found no opening between them. He noted, however, that the cytoplasm of the antheridium and the trichogyne became less dense, while the ascogonium furnished nucleated branches for ascus formation. He thought the antheridium was not functional.

Claussen (1905) published an excellent paper on this fungus, calling it *Boudiera*, under the impression that it was a new species of that genus, and not realizing that Van Tieghem had already given it the name *Ascodesmis nigricans*. Claussen at the beginning of his study referred material to Hennings (1903), who mistook it for a new species of *Boudiera* and described it under the name *B. Claussenii* P. Hen. n. sp. After the publication of Claussen's paper this error was corrected by Cavara (1905), who had previously seen the fungus growing on human excrement near Pavia and had published a record of his observation (1889). Cavara's note of correction has served to clear up any misapprehension concerning the identity of Claussen's fungus.

Claussen's paper describes the morphology of *Ascodesmis nigricans* in great detail. He showed how the spores germinate and rapidly form a mycelium of multinucleate cells. At numerous points on this mycelium rather complicated fruiting bodies are formed. Each of these consists at first of several oogonia and antheridia coiled about each other. At the tip of the oogonium is a trichogyne, and antheridium, oogonium, and trichogyne are all unicellular and multinucleate.

Claussen observed that an opening forms between antheridium and trichogyne, and later another forms, temporarily, between trichogyne and oogonium. The trichogyne nuclei were seen in stages of disintegration, and those in the antheridia disappeared, presumably migrating through the trichogyne into the oogonium, which was seen to have attained an increased number.

Stages in nuclear fusion were observed within the oogonium, first a pairing, then a dissolution of the contact membranes followed by a flowing together of the nuclear contents including the nucleoli. Larger nuclei appeared, which he interpreted to be the products of fusion.

Following this fusion, Claussen noted the withering of trichogyne and antheridium and the development from the oogonium of about two to four ascogenous hyphae. The ascogenous hyphae are short and unbranched, and each forms an ascus from its penultimate cell.

Each ascus at first contains two nuclei but these soon unite into one. Three successive nuclear divisions follow to form eight nuclei, which become included in as many spores by the process of free cell formation. He had great difficulty in finding nuclei in the act of division; in fact, he does not figure them at all. Likewise his figures of spore formation are meager, although the paper is otherwise beautifully illustrated.

Dangeard (1907) attempted to review Claussen's work. He failed to

find an opening between the antheridium and the trichogyne, and maintained that the antheridial nuclei disintegrate in situ. He questioned the validity of Van Tieghem's distinction between *A. nigricans* and *A. aurea*, holding that the latter is but an immature stage in the former.

In the matter of nuclear fusion in the oogonium, Claussen strangely reversed himself a few years later. In his study of *Pyronema confluens* (1906, 1907, 1912) he was unable to find such nuclear fusions there. Consequently he took the dangerous step of concluding, without further critical examination of *Ascodesmis nigricans*, that he had been in error as to its nuclear behavior, since two fungi so closely related should be alike in this respect. He thus introduced two possibilities of error, one in regard to *Pyronema confluens*, in which nuclear fusion in the oogonium had previously been described by Harper, and the other in regard to *Ascodesmis nigricans*, in which he had himself seen good evidence of such a fusion, and which might or might not agree with *Pyronema confluens*.

The situation in *Ascodesmis nigricans* was thus clouded, and has remained so since that time.

METHODS

Ascodesmis nigricans is not a commonly observed fungus. In the commercial production of mushroom spawn from spores, however, it has become something of a pest. Through the efforts of Dr. B. M. Duggar such a contaminated culture was obtained from Mr. J. F. Slyer, West Chester, Pa.

The writer isolated the fungus and grew it on rabbit dung agar made in the following manner. Three parts by measure of water and one of rabbit dung were placed in a large beaker and allowed to stand overnight in a refrigerator. The next day the mixture was brought slowly to boiling over an open flame and then heated a half-hour in the autoclave. The coarsest of the dung was then strained out with cheesecloth. Filtration was found to be very difficult, so the liquid was placed in a tall cylindrical graduate and kept in the refrigerator overnight. The next day the upper clear portion was removed from the lower turbid portion with a pipette. To each 100 cc. of rabbit dung infusion 3 grams of agar agar and 0.05 gram of dipotassium phosphate were added, and the material was heated in the autoclave for another 45 minutes. It was then tubed and autoclaved for 20 minutes.

The turbid portion of the agar was used for growing cultures for microtome sections, and the clear portion for whole mounts. Even with the sediment, however, the general contour of the agar sections on the slide was very difficult to see, and the lightly stained fruiting bodies were correspondingly hard to find.

To secure material for sections, a 10-cc. tube of rabbit-dung agar was melted and poured into a sterile Petri dish. When cool this was inoculated in the center with a few spores. The fungus growth penetrates the agar, extending in a circle from the point of inoculation. The fruiting bodies are mostly produced on the surface.

Except for some special work the cultures were grown in a nearly dark locker, at room temperature. To reduce evaporation, the culture dishes, usually four or five in a stack, were kept under a bell jar.

For microtome sections the material was fixed and washed in the dishes in which it was grown, and thus wilting and other disturbances incident to fixing were avoided. Several fixing fluids were tried, including weak and strong Flemming's solution, both full strength and diluted one-half with water. All of these gave excellent fixation as did Merkel's solution, although with the latter satisfactory staining was not so easy. Various times of fixation were tried from one to twenty-four hours. After a few trials an hour's fixation was regularly used.

After washing in running water, pieces of agar bearing the fungus were cut out for study. These were selected under a binocular microscope in obliquely reflected light to secure the maximum number of fruiting clusters on each piece. These pieces were run through ascending grades of alcohol and through cedar oil into paraffin, using the greatest care to avoid injury to the fruiting bodies. Their exposure, without ascocarps, on the surface of the agar insured quick penetration and fixation by the different reagents used.

At the outset some difficulty was experienced in keeping track of the sections owing to the transparency of both the agar and the fruiting bodies. This difficulty was overcome by adding a little lampblack to the tubes of agar just before sterilizing. This material had no effect on the development of the fungus, and as the fruiting bodies grew on the surface of the agar they were not surrounded by it. The lampblack made the sections very easy to find on the slide, and its use for general work of this character is recommended.

Both Flemming's triple stain and Heidenhain's iron-alum-haematoxylin were used. Good results were obtained with both, although the triple stain was preferred for the stages after the formation of the ascus.

Whole mounts were found best for studying early stages in the formation of sex organs. These were grown on dung agar directly on the slides where they were to be studied. They were kept in moist chambers until they had reached the desired stage of development, then fixed and stained with aceto-carmin, Ehrlich's haematoxylin, or a combination of the two, and a few in iron-alum-haematoxylin.

OBSERVATIONS

Ascodesmis nigricans is coprophilous, growing readily on the dung of various animals and on culture media made from such materials. On standard beef agar it grows moderately well, but fruits only scantily. It is homothallic, developing perfectly in cultures from single-spore isolations.

The spores germinate readily on rabbit dung agar, forming an approximately circular radiating growth. The mycelium is septate, with multinucleate cells (fig. 1, pl. 1). The fungus forms ascospores freely, but no conidia have been found. Some fifty to sixty hours after spore germination, under the most favorable conditions, the sexual organs begin to form.

Antheridia and oogonia may be formed on the same branch (fig. 2, 3, pl. 1) or on neighboring branches. They start as knobs which elongate into stalks that branch dichotomously, and, growing toward each other, their ultimate branches intertwine, an oogonial branch with an antheridial one (fig. 3, pl. 1). Commonly there are four to eight coiled pairs in a cluster supported by two stalks, one oogonial and one antheridial (fig. 4, pl. 1). Mature antheridia are one-celled structures on a dichotomously branching stalk. Mature oogonia are similar in appearance but each has a unicellular trichogyne at the tip. The antheridia, oogonia, trichogynes, and stalks are all multinucleate.

The nuclei of the trichogyne disintegrate in situ before any opening takes place in its wall. An opening then forms between the antheridium and the trichogyne (fig. 5, pl. 1). It is not difficult to demonstrate this in whole mounts. Claussen (1905) reports having seen it thus very many times, "mehr als tausendmal," but only a few times in sections. The actual passage of the antheridial nuclei into the trichogyne has not been observed, but the fact that they disappear from the antheridium without disintegration leads to the conclusion that they do so migrate, especially as *Ascodesmis nigricans* gives every evidence of normal sexuality, rather than of any of the various forms of sexual degeneration or modification found in some other Ascomycetes. Claussen (1905) observed also an opening in the septum separating the trichogyne from the oogonium. This the writer has not seen. As in *Pyronema confluens* (Harper, 1900) and in some other Ascomycetes, it closes soon after the passage of the antheridial nuclei, and hence its observation becomes partly a matter of chance.

Each fruiting cluster contains numerous coils of conjugating pairs, and as the ascogonia develop they are much crowded and change considerably in size and shape. They become septate and some cells may enlarge to several times their original diameter. The antheridia and the trichogynes wither (fig. 11, 13, 17, pl. 1), and the ascogonia develop ascogenous hyphae into which pass most of the cytoplasm and nuclei, leaving them nearly empty (fig. 24, pl. 2). These ascogenous hyphae do not grow out simultaneously, but the first may be far in advance of the last. By the time the last are starting, asci may be forming at the tips of the older ones. The ascogenous hyphae are rather short, relatively straight, and unbranched.

The question of the behavior of the nuclei from the time of fertilization to the time of ascus formation has become so controversial in the different species of Ascomycetes studied that the writer has given particular attention to its investigation in this study of *Ascodesmis nigricans*. At the moment of fertilization the difference in size between the antheridial and the oogonial nuclei is not great. In each group they vary slightly, but those from the oogonium average somewhat larger. There are only a few of each, and their behavior is easy to follow.

Within the ascogonium, which consists of from one to three cells, these nuclei show no definite distribution. Some are isolated, but pairs are not

uncommon (fig. 5, 6, 7, 8, 16, pl. 1; fig. 22, 23, pl. 2). The writer has seen every stage in nuclear fusion from pairs with membranes intact (fig. 5, 6, 7, 8, pl. 1; fig. 23, pl. 2) to the last stage in nucleolar fusion (fig. 20, pl. 2) and some nuclei fully twice the size of others (fig. 21, pl. 2). The nuclei pair in the resting condition, the adjacent membranes break down, the contents flow together into a common membrane forming a large oval nucleus with two nucleoli, and these finally unite, after which the fusion nucleus becomes nearly spherical. Stages in this fusion have been seen many times by the writer, and there can be no doubt of its occurrence in *Ascodesmis nigricans*.

The writer has had in mind all the pitfalls that have been mentioned by those who oppose the idea of a nuclear fusion in the ascogonium. In well-stained material the density of the cytoplasm does not interfere with vision, and it is possible to distinguish between nuclei in mere contact and nuclei whose adjacent membranes are disappearing. The best apochromatic lenses have been used in this study. Inevitably a large proportion of nuclear pairs will be so oriented that one will be more or less above the other on the slide. All such have been rejected from the evidence, although by careful focusing the writer was convinced in many cases that actual fusion was taking place. All drawings were made from nuclei in practically the same plane of focus excepting figures 23, 24, and 29, which were drawn for other purposes, and in which the superposition is plainly shown.

While the writer is convinced that nuclear fusion takes place in the ascogonium of *Ascodesmis nigricans*, absolute proof that one member of each fusing nuclear pair is antheridial and the other oogonial is very difficult to produce. In the first place the antheridium and the oogonium each contribute several nuclei but apparently not a fixed number. Furthermore the difference in size between the two sets of nuclei is not marked and is overlapping. Figures 13, 14, 16, and 17 show one of the nuclei (presumably the female) to be larger than the other, but in figures 9, 10, 11, 12, and 18 the two are practically equal. In all likelihood, however, these fusions are between male and female nuclei, for a fusion between two male or two female nuclei when both are present in the same cell, and in a plant where there is every evidence of normal sexuality, is not to be expected.

Likewise it is difficult to prove that each of the nuclei that unite to form the definitive nucleus of the ascus is one of those resulting from fusion in the ascogonium, or its descendent. The best evidence that such is the case lies in the fact that no nuclear division seems to take place in the ascogonium or in the ascogenous hyphae (before crozier formation), and the number of asci formed is about sufficient to account for the available nuclei if all or practically all the antheridial and oogonial nuclei united.

Something should be said here as to the structure of the nuclei in *Ascodesmis nigricans*. For the most part they are round or slightly oval, each with a single large, round, smooth nucleolus. Prior to ascus formation the chromatin forms a scanty and rather uniform deposit between the nucleolus

and the nuclear membrane. In the ascus the nucleus grows rapidly, some enlargement taking place before fusion. At the time the definitive nucleus is ready to divide, the chromatin forms a distinct and rather even reticulum (fig. 32, pl. 2).

Each ascogenous hypha receives two nuclei, rarely three or four. These may be antheridial, oogonial, or fusion nuclei. Not infrequently, the ascogonial nuclear fusion is completed in the ascogenous hyphae (fig. 16, pl. 1; fig. 24, pl. 2). The ascogenous hyphae are short, simple, and easily studied. In dozens of cases the writer has seen entire ones in a single section. Each is cut off from the ascogonium by a basal septum. It then develops a crozier at its tip in the manner so often described for various Ascomycetes. The writer has never observed fusion between the tip cell and the third or stalk cell to form a second ascus. Certainly such a union does not always take place, for disintegration of the nucleus of the tip cell was observed in a few cases, and Claussen (1905) reports seeing (as the writer has twice) a well-developed ascus with an isolated tip cell adhering to it.

The ascus develops normally from the penultimate cell. It receives two nuclei which usually do not fuse until the ascus has enlarged considerably. Figures 30 and 31 show the size of the ascus when a fusion of its nuclei most commonly occurs. Following this fusion the ascus continues to grow for some time (probably an hour or so), and then the nucleus quickly undergoes three successive mitotic divisions to form eight nuclei, which migrate to the periphery of the ascus. After another short pause eight spores are formed about these nuclei by the process of free cell formation.

The writer has not, as yet, determined the chromosome number in *Ascodesmis nigricans*, much less the details of meiosis or brachymeiosis. The nuclei appear to divide with great rapidity, even for Ascomycetes, so that mitotic figures are few, even in sections where stages just before and just after division are numerous.

The ripe ascospores of *Ascodesmis nigricans* are beautiful structures. They are spherical or nearly so with a highly sculptured exospore. This sculpturing consists of prominent ridges bounding polygonal areas—usually hexagons. Each spore has but a single nucleus throughout its development.

As the spores ripen, the ascus greatly elongates and finally it discharges its spores in a mass to a distance of several centimeters. Apparently this fungus has no elaborate mechanism for the discharge of its spores. The end of the ascus seems to be blown off by internal pressure due to absorption of water. The spores require little or no rest period before germination. They remain alive in old cultures in the refrigerator for at least two years.

In *Ascodesmis nigricans* there is no well-developed ascocarp (fig. 33, pl. 2). Numerous paraphyses grow from the stalks bearing the sexual coils, but none from the ascogenous hyphae. The outer paraphyses cover the asci to some extent, but seem not essentially different from the others. They are nearly cylindrical and septate, and have very conspicuous bodies in their terminal cells.

DISCUSSION

It is evident from this investigation and those that have preceded it that generalized statements regarding the Ascomycetes are treacherous, and that only within narrow limits, and with readiness to allow for exceptions, can one predict from the structure or physiology of one species what will be found in another.

Nevertheless, to bring order out of chaos, it is desirable to discover certain guiding principles, and by detailed investigations to learn the extent to which each species follows these principles or deviates from them. It is known, for example, that sexual reproduction represents a general tendency among the Ascomycetes. In all likelihood there was a time when all the species then existing possessed it. Evolutionary tendencies arising independently in different parts of the class, and at different times, have brought about many modifications of it and even its total loss. Thus we have now well-known cases of isogamy, apogamy, and probably also pseudomixis and parthenogenesis.

Perhaps the most perplexing, and certainly the most vexing, question is that concerning the repetition of nuclear fusion within a single life cycle, a fusion in the ascogonium followed by a fusion in the ascus. That it occurs in some groups, notably certain Erysiphales and Pezizales, is proved as conclusively as the cytological methods of today will permit. The extent of its occurrence throughout the class can be determined only by the most painstaking research involving a large number of species, and done by competent cytologists.

It seems wise here to take account of the different lines of evidence used to prove or to disprove nuclear fusion in the ascogonium. Admitting that it contains two kinds of nuclei from the cells that have united, either by true fertilization or by some modification of it, there are in vogue four lines of observation to determine whether or not these nuclei fuse.

(1) Nuclear pairing has been used both to prove and to disprove fusion. Finding nuclei together, it is easy to assume that they were about to unite. It is likewise easy to assume that these pairs are synkaryons such as are found in the Basidiomycetes. Neither assumption constitutes dependable evidence, although in a given case, either may happen to represent the fact.

(2) The observation of stages in nuclear fusion is often reported. Admittedly in fixed material fusion as an active continuous process does not take place. The nearest that can be found is a series of nuclear pairs, which if arranged in proper sequence, would represent stages in fusion. In well-fixed and stained material this constitutes powerful evidence. Poor material may contain artifacts that simulate such unions, and nuclei more or less superposed in the plane of focus are liable to be misleading, especially if they are very small. An experienced cytologist, however, will recognize these pitfalls.

(3) Nuclear numbers are useful in fungi with uninucleate cells. If a uninucleate oogonium becomes binucleate by the acquisition of an antheridial

nucleus, and at a later stage it again becomes uninucleate, the evidence is strong that a nuclear fusion has taken place. The possible alternatives are that the cell has again become uninucleate by septation or by the distintegration of one of the nuclei. If, on the other hand, uninucleate gametes unite and this union is followed by a regularly binucleate condition, there is good evidence that nuclear fusion has not occurred. The alternative is that a fusion has taken place followed by mitosis.

In coenocytic cells, nuclear counts are not likely to yield conclusive evidence, as the counts cannot generally be made with accuracy. This is especially true if the number of nuclei is very large. In case mitosis intervenes, this line of evidence becomes very treacherous.

(4) Chromosome counts are of great value when they can be made with certainty. The change from a haploid number to a diploid number presupposes a union of haploid nuclei. The occurrence of a tetraploid number presupposes a union of diploid nuclei. The accepted method of determining whether a nucleus is haploid, diploid, or tetraploid is to determine the number of chromosomes that enter or leave it during mitosis. According to most investigators, meiosis takes place during the first one or two mitoses in the ascus. A further reduction in number in the second or third mitosis has been recorded by several investigators, and for this process the term brachymeiosis was proposed by Fraser (1908).

The determination of the existence of brachymeiosis is not easily carried out. The nuclei are relatively small, and in some species their division takes place very quickly. Furthermore, the chromosomes may remain for a time in pairs instead of separating during division, thus forming "bivalent" chromosomes, as has been shown particularly well in the relatively large nuclei of *Phyllactinia Corylea* (Pers.) Karst. (Harper, 1905). In many ascus nuclei, however, the chromosomes are so small and so tightly pressed together that a conjugating pair cannot be distinguished from a single one. Hence their valency, and correspondingly their number, may be disputed. It is only fair to say for this line of evidence that in so far as chromosome counts can be made with accuracy they serve as a strong indication of the number of nuclei that have united.

To the four lines of evidence just discussed may be added a fifth—nuclear size. A relatively large nucleus may or may not be a fusion nucleus, for nuclei grow and divide and thus change their size in ways other than by union. This line of evidence has value only when properly safeguarded.

An examination of the table beginning on page 521 shows where these several lines of evidence have been used in about sixty separate researches by more than thirty investigators. Negative evidence is also used, but it is everywhere recognized as weak, even though it sometimes represents the facts.

This table also shows that there are two strongly supported conceptions regarding the nuclear behavior in the sexuality of the Ascomycetes.

(1) The idea that two nuclear fusions take place in the life history of

certain species was first advanced by Harper (1895) for *Sphaerotheca Humuli* (DC) Burr. It is supported on strong evidence by several investigators of this and other species. This conception labors against one great handicap, largely psychological. It is agreed without reservation that nuclear fusion takes place in the ascus. The details of nuclear fusion in the ascogonium are closely similar and probably would be accepted with equal confidence were it not that two nuclear fusions in each life cycle are not what one would expect from a knowledge of other plants, and so there is a reluctance on the part of investigators to adopt the idea of two fusions in one life cycle of a fungus. Some other alternative, therefore, is constantly being sought.

(2) The idea that there is a nuclear pairing in the ascogonium followed by only one nuclear fusion, that in the ascus, was first suggested, according to Gwynne-Vaughan (1922, page 45), by Raciborski in a letter to Harper written in 1895 and published the following year. Maire (1903) adopted this view in its application to *Galactinia succosa* Berk., where the ascogenous hypha consists of a short filament of binucleate cells. He called this structure a synkaryophyte, but the term has not been widely adopted. This conception got its greatest impetus from Claussen (1912) through his description and figures of *Pyronema confluens*. This coenocytic fungus is not a favorable one, however, for the establishment of such a hypothesis, and more convincing evidence comes from Wieben (1927) who, working on *Taphrina*, found that the "conidia" that bud from the ascospores conjugate in pairs to form a mycelium with binucleate cells. Her observation on conidial conjugation is supported in a note by Mix (1929).

This hypothesis of a single nuclear fusion in the life cycle, following a prolonged nuclear pairing, is the more easy of acceptance because of the somewhat similar condition that exists in the Basidiomycetes.

As a third alternative the writer attaches little importance to the contention of Dangeard and Moreau, offered in defense of a lost cause, that there is no entrance of antheridial nuclei into the oogonia of such forms as *Pyronema confluens* and *Sphaerotheca Humuli*, and consequently no opportunity for a sexual nuclear fusion. Such an extreme view offered in the face of overwhelming evidence must inevitably perish with its authors.

Dodge (1920, 1927, 1928a, 1928b, 1931a) and Shear and Dodge (1927) have attacked the problem of sexuality in the Ascomycetes from an entirely new angle. Bringing together different strains of heterothallic *Neurospora* species, Dodge has demonstrated beyond question the hybrid nature of the offspring. As these facts are quite damaging to the contention of Dangeard and Moreau that there is no true sexuality in most Ascomycetes, the latter (1930) has sought to combat them with a weird hypothesis which does not admit any nuclear fusion but rather presumes that anastomoses between vegetative hyphae of the two strains permit a mixing of cytological elements, whose physical character is not designated, to form the hybrid offspring. The hybrid character of the perithecium is either a result of such anastomoses,

or of the union in their tissues of filaments taken from the two parents—"des périthèces puissent acquérir des apparences de périthèces hybrides dans les cultures mixtes d'espèces différentes, soit à la suite de telles anastomoses, soit par la réunion, dans leur tissus, de filaments empruntés aux espèces en présence."

This seems like trying to shape the facts to a preconceived theory rather than to formulate a theory in harmony with the facts. It is not likely that the laws governing the transmission of heredity, based on a century of careful work, can be so lightly brushed aside. If, as Moreau says, the cells of the ascogenous hyphae are uninucleate, this is evidence that their nuclei must be the products of fusion as they carry the hereditary qualities of both parents into the nuclei.

More recently Moreau and Moruzi (1931) have published the results of growing different strains of heterothallic species of *Neurospora* in opposite ends of a U-tube of culture medium. Under these conditions they report obtaining fertile asci in one arm although they deny physical contact between the two strains. They assume that a hormone or its equivalent ('harmozone') diffusing from one arm of the U-tube to the other has the effect of an act of fertilization. Dodge (1931b), however, by this method obtained contradictory results in that ascospores were not formed in such U-tube cultures if there was no contact between the mycelia of the two strains of *Neurospora*.

With two nuclear fusions in the life cycle, as is clearly the fact in some species of Ascomycetes, two questions arise. Which should be interpreted as the sexual fusion, and what is the explanation of the occurrence of the other? On the first point it seems generally agreed that the ascogonial fusion is the sexual one, as it takes place between nuclei more or less unlike and as it is at the beginning of the sporophytic generation. Some efforts have been made to answer the second question as to the cause of fusion in the ascus, but none of them are wholly satisfactory.

Where the sporophytic generation is shortened to the extreme, as in *Dipodascus*, *Endomyces*, *Eremascus*, and *Laboulbenia*, a single nuclear fusion seems to serve every purpose, and the evidence is strong that only one occurs. Whether it shall be classed as the "ascogonial fusion" (see fourth note, page 524) or the "ascus fusion" seems purely a matter of definition so long as it is recognized as a sexual fusion.

Indeed, the reduction has gone a step further in *Thielavia Sepedonium*, where Emmons (1932), in a careful study, has found no nuclear fusion in the entire life cycle, not even in the ascus.

Probably the time has arrived when we should abandon the idea that all Ascomycetes behave alike in regard to the number of nuclear fusions that take place in one life cycle and that all those investigators that do not agree are in error. The evidence is convincing that there are two successive fusions in *Ascodesmis*, *Erysiphe*, *Humaria*, *Lachnea*, *Phyllactinia*, *Pyronema*, and *Sphaerotheca*. It is equally convincing that there is only one fusion in

Dipodascus, *Endomyces*, *Eremascus*, *Laboulbenia*, *Taphrina*, and *Thielavia Sepedonium*.

Perhaps some lack of uniformity in nuclear behavior should be expected in a group of fungi showing such diversity in the morphology of their sexual apparatus. Nowhere in the plant kingdom is there another group with such a wide variation in the detail of sexuality as exhibited by the Ascomycetes. There are multinucleate sex organs (*Ascodesmis*, *Ascobolus*, *Dipodascus*, *Lachnea*, *Pyronema*), and uninucleate (*Endomyces*, *Eremascus*, *Erysiphe*, *Phyllactinia*, *Polystigma*, *Sphaerotheca*). There are multicellular oogonial structures (*Ascophanus*, *Ascobolus*, *Rhizinia*) and unicellular (*Ascodesmis*, *Dipodascus*, *Erysiphe*, *Monascus*, *Phyllactinia*, *Pyronema*, *Sphaerotheca*). There are species with functional trichogynes (*Ascodesmis*, *Monascus*, *Pyronema*, *Venturia*), non-functional ones (*Laboulbenia*, *Lachnea*, *Polystigma*), and none at all (*Dipodascus*, *Erysiphe*, *Helvella*, *Phyllactinia*, *Taphrina*). A few produce spermatia, functional or otherwise (*Collema*, *Polystigma*, *Rhytisma*), but most species do not. Some have simple ascogenous hyphae (*Ascodesmis*, *Sphaerotheca*), some branching ones (*Erysiphe*, *Pyronema*), and some none at all (*Dipodascus*, *Eremascus*). Nuclear division occurs in some ascogenous hyphae (*Lachnea*, *Pyronema*), but in others none has been observed excepting at the tip (*Ascodesmis*, *Mycosphaerella*). The ascus commonly forms from the penultimate cell, but even in the same species or individual many exceptions have been found.

Normal sexuality is common in some (*Ascodesmis*, *Endomyces*, *Erysiphe*, *Monascus*, *Phyllactinia*, *Pyronema*, *Sphaerotheca*), is variously modified in others (*Ascobolus*, *Ascophanus*, *Helvella*, *Humaria*, *Lachnea*, *Polystigma*, and *Taphrina*), and is completely suppressed in still others (most of the Fungi Imperfecti).

With this array of differences known to exist in the sexual structures of the Ascomycetes, and with the many careful researches on the nuclear phenomena, the position that all Ascomycetes have two nuclear fusions in their life histories, or that none of them have two, seems untenable.

SUMMARY

1. Much difference of opinion exists among cytologists concerning the occurrence of a nuclear fusion in the ascogonium of the Ascomycetes.
2. *Ascodesmis nigricans* is a close relative of *Pyronema confluens* in which this controversy has reached its climax.
3. *Ascodesmis nigricans* was carefully investigated by Claussen in 1905, who then reported a normal fertilization, with a nuclear fusion in the ascogonium, followed by a second in the ascus.
4. In 1907 Claussen reversed his decision concerning nuclear fusion in the ascogonium of *Ascodesmis nigricans* because he could find none in its near relative *Pyronema confluens*.

5. *Ascodesmis nigricans* has been reinvestigated by the present writer with confirmation of Claussen's earlier findings on the morphology and cytology of this fungus.

6. Numerous cases of nuclear fusion both in the ascogonism and in the ascus of *Ascodesmis nigricans* have been described and illustrated.

7. The nuclei unite in the resting condition. The adjacent membranes dissolve away and the contents flow together, the nucleoli fusing last.

8. An analysis has been made of the kinds of evidence used by different cytologists for and against nuclear fusion.

9. A table has been prepared summarizing nearly sixty pieces of research on the sexuality of different Ascomycetes.

10. The opinion is expressed that the controversy over this question is due in part to faulty observation, and in part to actual differences existing among the species investigated.

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EXPLANATION OF PLATES

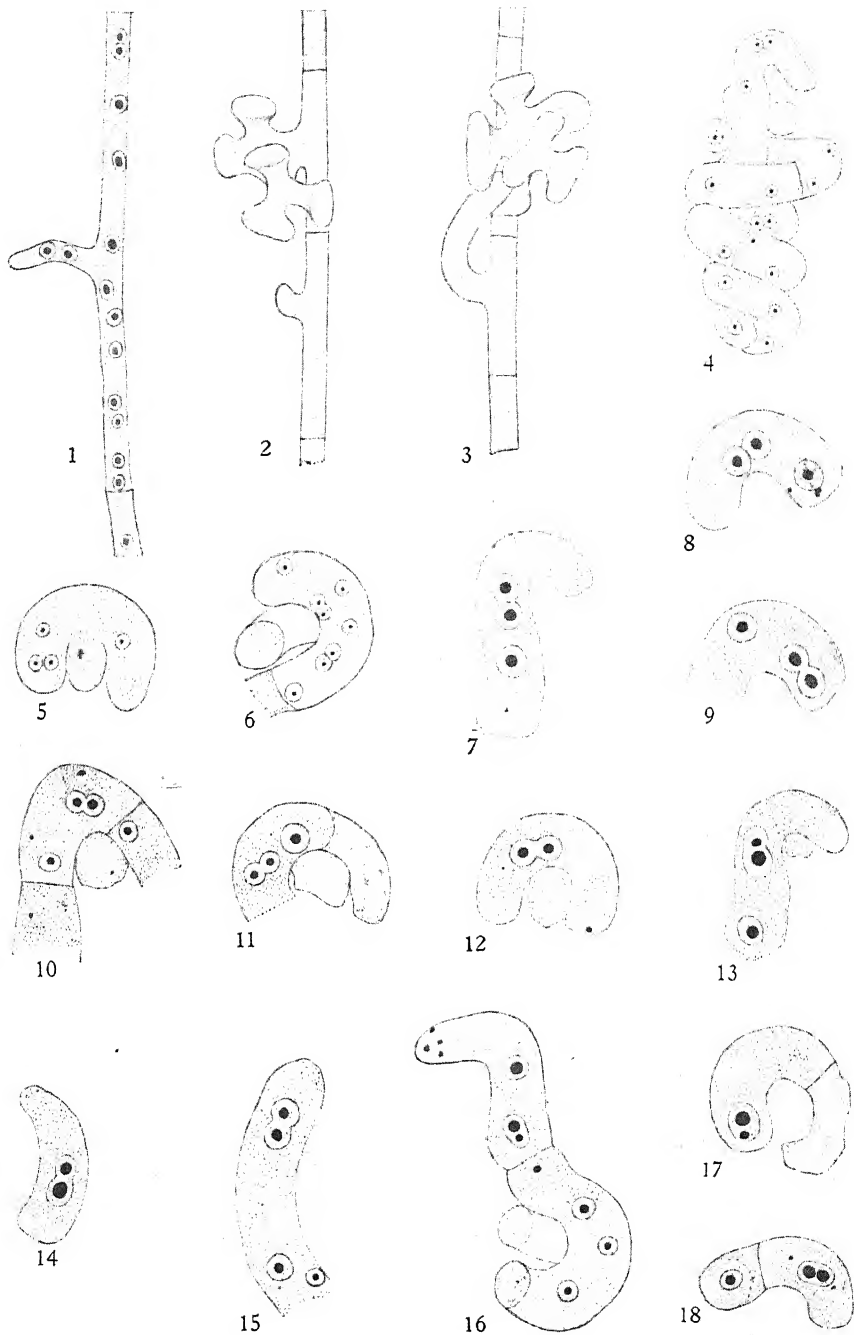
All figures were drawn with the aid of a camera lucida, using a Zeiss 2 mm. apochromatic objective, 1.40 numerical aperture and compensating oculars. Figures 1, 2, 3, and 33 are magnified 1100 times, figure 4 is magnified 1600 times, and figures 5 to 32 are magnified 2600 times.

PLATE 1

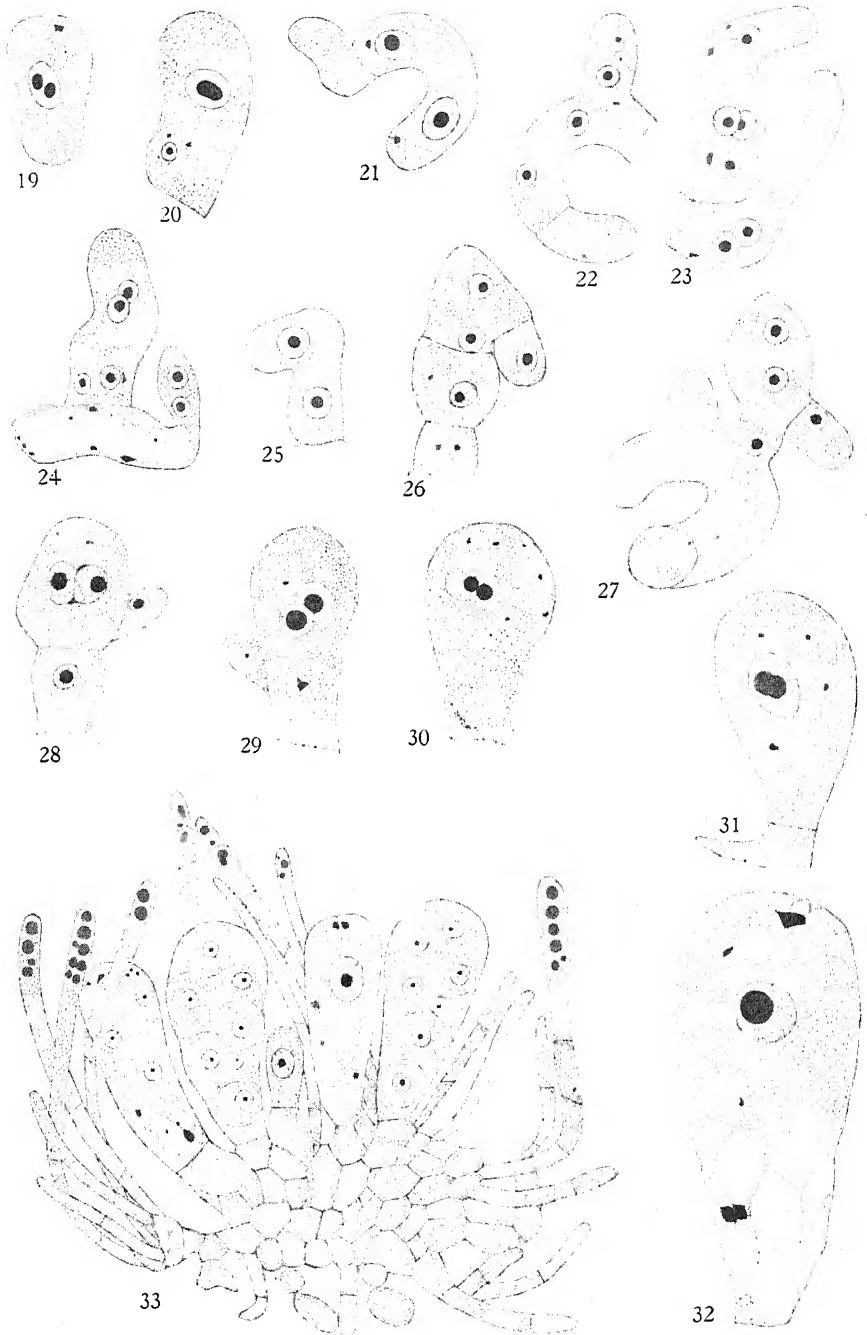
- Fig. 1. Portion of hypha showing coenocytic condition.
- Fig. 2. Primordia of oogonia and antheridia branching from the same hypha. External view.
- Fig. 3. Primordia of oogonia and antheridia beginning to associate with each other. External view.
- Fig. 4. Two coiled pairs of oogonia and antheridia.
- Fig. 5. Opening between antheridium and trichogyne.
- Fig. 6. Ascogonium, shortly after fertilization, coiled about empty antheridium.
- Fig. 7, 8. Ascogonia with nuclei paired, nuclear membranes intact.
- Fig. 9-20. Stages in nuclear fusion in ascogonia and ascogenous hyphae.
- Fig. 9. Ascogonium containing a pair of nuclei in which the membranes are beginning to disintegrate at the point of contact.
- Fig. 10-13. Similar to fig. 9 but with nuclear fusion further advanced. Withering antheridium in the curve of the ascogonium.
- Fig. 14, 15. Portions of ascogonia, each showing one pair of fusing nuclei.
- Fig. 16. Ascogonium and ascogenous hypha. Withering antheridium in the curve of the ascogonium.
- Fig. 17. Portion of terminal cell of ascogonium with shriveled trichogyne. Nuclear fusion nearly complete.
- Fig. 18. Portion of ascogonium with nucleoli ready to fuse.

PLATE 2

- Fig. 19. Portion of ascogonium with nucleoli ready to fuse.
- Fig. 20. Portion of ascogenous hypha with nucleoli nearly fused.
- Fig. 21. Ascogonium with two large nuclei, presumably the products of fusion.
- Fig. 22, 23. Ascogonia, each with an ascogenous hypha branching from it.
- Fig. 24. Ascogonium with two ascogenous hyphae. In the larger, nuclear fusion is not yet complete. One nucleus is in a slightly higher plane of focus than its mate.
- Fig. 25. Ascogenous hypha bending to form "crozier."
- Fig. 26. Typical "crozier" in ascogenous hypha.
- Fig. 27. Ascogonium with ascogenous hypha producing an ascus.
- Fig. 28-31. Young asci showing stages in nuclear fusion.
- Fig. 32. Uninucleate ascus not quite full grown.
- Fig. 33. Section of young fruiting cluster showing asci in different stages of development—also paraphyses.



SWINGLE: ASCODESMIS NIGRICANS



SWINGLE: ASCODESMIS NIGRICANS

DEPTH STUDIES ON PHOTOSYNTHESIS OF THE RED ALGAE ¹

R. H. TSCHUDY

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The red algae, because of their ability to grow in relatively deeper waters than the green and brown algae, present several questions of considerable interest: First, what part does the red coloring matter of these algae, phycoerythrin, take in the synthesis of carbohydrates? Second, what is the chemical nature of phycoerythrin? The purpose of this paper is to present evidence that photosynthesis takes place in the red algae at depths where very little light of the longer wave lengths commonly used in synthesis by other chlorophyll-bearing plants penetrates, and to discuss the various factors such as clouds, water motion, etc., which have a bearing on plant life at depths where these plants grow.

The red algae first began to attract the interest of plant physiologists when Pringsheim (1874), Sorby (1877), Reinke (1886), and Molisch (1894) began work on the nature of the red coloring matter. About this time Engelmann (1882) started working on photosynthesis with his oxygen bacterium method. He presented evidence that the color of the alga is complementary to that of the incident light which penetrates to the depth at which the alga grows. He also showed by projecting the microspectrum on plants that most oxygen was produced in the red spectral zone in the case of the green algae, and in the green spectral zone in the case of the red algae. He believed that the brown and red pigments also play a part in synthesis for this reason, and applied the name chromophylls to the group of photosynthetic pigments.

Sporadic work of little importance was continued from time to time by various workers, but it was not until Hanson (1909) published the results of his efforts that any real advance was made in the study of the physiology of the red algae. In this paper he mentioned that the red algae grow at a depth at which very little light penetrates, particularly that which is most effective in photosynthesis, and that these algae also contain chlorophyll in addition to the red pigment. He suggested that the phycoerythrin absorbs the energy from the light which reaches these depths, and passes it on for the chlorophyll to use in synthesis. He says, "From the deep orange fluorescence (of the red coloring matter) it is conjectured that phycoerythrin absorbs the blue-green light and degrades it to yellow and red which can be absorbed in turn by the chlorophyll." It is interesting to note in this connection that

¹ Contribution from the laboratories of Botany and Oceanography, University of Washington.

Engelmann has shown that chlorophyll itself, in the presence of blue-green wave lengths, may absorb the light and produce photosynthesis.

A paper by Moore, Whitley, and Webster (1922) attempts to show relationships between light intensity and depth, as well as color of light and photosynthetic activity. The authors found that in the bright sunshine the red algae synthesized much less rapidly than the green, and in diffused light the reds synthesized more rapidly than did the greens. They also found that the rate of synthesis of the reds in diffused light was as great as, or greater than, in bright light. They conclude, as Engelmann does, that the red pigment does not act as a passive color screen, but that the pigments of the red and brown algae play a positive part in synthesis.

In the work by Moore, Whitley, and Webster, different colored spectral glasses were placed over pie plates containing the algae. The number of cc. of centinormal alkali necessary to neutralize 100 cc. of the sea water in which the algae had been immersed was used to determine the amount of photosynthesis. The temperature of the sea water in the tins seems to be a factor that is overlooked by these workers; at any rate it is not mentioned. This omission leaves the question unanswered as to whether the results observed were due to differences in light or differences in temperature.

Shelford and Gail (1922) found that the red algae begin at depths at which the red and orange light is reduced to about 1 per cent and extend to where the red light is approximately 0.0032 per cent of the incident light. "They are most abundant where the shorter wave lengths are approximately 2.9 per cent. The depth at which the red algae grow, when compared with these wave lengths, probably gives a clue to the reason for the red color of the algae growing in deeper water." It seems to me that the authors are assuming too much of a causal relationship between the red color and the wave length of light.

Following this, Gail (1922) worked on the depth at which maximum photosynthesis takes place in some of the algae of the Puget Sound region.

Gail placed the algae in small glass-stoppered bottles and then immersed the bottles in the sea. The temperature was thus made constant. He then tested for oxygen by means of the Winkler method.

METHOD AND APPARATUS

Dr. Lund, in class work at the Puget Sound Biological Station in 1930, used calibrated test tubes in shallow water. I have used this method with a large measure of success in work at greater depths.

The apparatus consisted of wire baskets made of about 3/16 inch iron wire with meshes 1 inch square and the whole galvanized. The calibrated tubes were fastened horizontally in the bottom. The tubes were about 8 inches long, 1 inch in diameter, and held about 80 cc. The advantage in using these tubes instead of glass-stoppered bottles is obvious. Pieces of alga 2×15 cm. can

be cut from a frond and readily inserted or removed. A one-hole rubber stopper with a cone cut out of the under surface to permit the bubbles of air to escape is inserted to the calibration mark, and then the hole is stopped by a short, tapered piece of glass rod. The tubes are held in place horizontally on the bottom of the basket by means of a piece of gum rubber tubing threaded up and down through the meshes. This permits the tubes to be placed in position or removed rapidly and conveniently, gives the fronds minimum interference from the rays of the sun, and holds the tubes firmly in position. The baskets were about a foot square and were suspended by their four corners by means of short pieces of galvanized wire attached to a central ring. This was in turn tied to a rope. The lower end of the rope was fastened by an anchor and the upper end held in position by a float.

Due to the fact that some of the brown algae grow at the same depths as do the reds, notably *Nereocystis* in its younger stages, it was thought that parallel experiments should be run to determine the photosynthesis of brown and red algae under identical conditions of temperature, depth, time of day, amount of illumination, duration of experiment, and area of tissue exposed.

Experiments were at first tried using the same time that Gail used in his experiments, but it was found that in half an hour sufficient photosynthesis or respiration to give reliable results had not taken place. In most of the significant work done in the summer of 1931, the experiments were allowed to run eight hours. In 1932 it was found that no reliable results could be obtained in less than four hours' exposure.

The tubes were cleaned, rinsed in sea water, and the fronds cut. Water for filling the tubes was collected at some distance from the laboratories and from the town, due to the danger from sewage pollution. A five-gallon carboy was used to contain the sea water. The carboy was pushed under the surface from the back of a rowboat, and a glass tube $\frac{1}{2}$ inch in diameter was inserted through the mouth so that air could escape without bubbling and thus mixing too much with the sea water. The carboy was then rolled so that the water would become homogeneous. A siphon was attached so that water was taken from the bottom of the jar and not from the water in contact with the air.

The tubes and fronds were then rinsed with this water. The pieces of fronds were inserted in the tubes, rinsed once while in the tube; the tubes were then filled to overflowing with water, and the previously moistened stopper was inserted. Care was taken to exclude all air bubbles. A blank control of sea water was placed in each basket in every experiment, and a blank was also taken at the time that the tubes were filled, and analyzed for oxygen immediately. The Winkler method was used for the determination of oxygen. Thompson and Robinson (1932) state that the Winkler method is universally used for the determination of dissolved oxygen, and that with proper care of reagents the concentration of dissolved oxygen may be determined with marked accuracy.

TABLE 1. *Oxygen evolved at different depths, and also the weather conditions and condition of the water on the days that the experiments were run. In all tables results are expressed in ml. of oxygen present in 50 ml. of solution.*

	1	2	3	4	5
	6-11 a.m. Clear, smooth, sunny	2-5 p.m. Clear, smooth, sunny	6 a.m.-3 p.m. Slightly cloudy, calm	6 a.m.-2 p.m. Calm, fairly dark clouds	6 a.m.-2 p.m. Calm, half cloudy
5 meters					
Blank092	.129	.203	.251	.194
<i>Nereocystis</i>388	.246	.725	1.139	.721
<i>Iridaea</i>314	.326	.920	1.19	.592
<i>Turnerella</i>345	.350	.832	.980	.775
15 meters					
Blank073	.123	.222	.240	.185
<i>Nereocystis</i>204	.117	.101	.086	.444
<i>Iridaea</i>216	.166	.407	.554	.586
<i>Turnerella</i>185	.138	.382	.382	.431
25 meters					
Blank104	.102	.205	.240	.169
<i>Nereocystis</i>071	.055	.074	.062	.142
<i>Iridaea</i>104	.092	.148	.179	.160
<i>Turnerella</i>074	.135	.098	.145	.166
Blank run before starting experiment	.126	.086	.191	.246	.204

It is evident in columns 1 and 2 of table 1 that the results are unreliable, because there is not a sufficient difference between the experiments and the water controls. This is due to the short time that the experiments were run. The remainder of the table shows that though most of the photosynthesis seems to occur at shallower depths, the red algae utilize light at greater depths than do the brown algae. The controls of sea water, which contain more oxygen than do the tubes containing brown algae, suggest that at 25 meters and in most cases at 15 meters, respiration rather than photosynthesis is taking place at the more rapid pace.

In order to interpret correctly the results of these experiments, the data of an experiment on respiration must be consulted (table 2).

TABLE 2. *Oxygen content of tubes in light and total darkness*

Water control at beginning of experiment244	
<i>Nereocystis</i> in dark 6 hours108	.086
<i>Nereocystis</i> in light 6 hours086	.875
Water control after 6 hours in light252	

The tubes containing *Nereocystis* were run in duplicate. The tubes in the dark were encased in black paper to exclude the light. This table shows that the amount of oxygen in the water to begin with was .244 ml. After being in the light 6 hours, this amount increased slightly, to .252 ml., due to the photosynthesis of diatoms, etc., in the sea water itself. *Nereocystis* strips

in the light produced oxygen, as shown by the figures, while those in the dark used it up in respiration.

It will be noted that the respiration figures correspond to the values found for brown algae at depths of 15 meters or greater. Likewise in the other tubes—i.e., those containing red algae and used in tables 1, 3, 4, and 5, and suspended at a depth of 25 meters—respiration has taken place at a greater rate than photosynthesis. In all probability, in most cases respiration has been the only process and photosynthesis has been entirely inhibited.

TABLE 3. *Ml. of oxygen produced by Nereocystis, a brown alga, and Opuntiaella and Schizymenia, red algae, at different depths*

	1	2	3
	6 a.m.-4 p.m. Clear some- what choppy	6 a.m.-3 p.m. Clear, calm	6 a.m.-4 p.m. Partly cloudy, choppy
	5 meters	5 meters	5 meters
Blank216	.160	.179
<i>Nereocystis</i>536	.145	.747
<i>Schizymenia</i>592	.599	.420
<i>Opuntiaella</i>525	.400	.554
	15 meters	15 meters	15 meters
Blank191	.166	.148
<i>Nereocystis</i>083	.454	.135
<i>Schizymenia</i>462	.425	.508
<i>Opuntiaella</i>188	.348	.447
	25 meters	22.5 meters	22.5 meters
Blank191	.240	.202
<i>Nereocystis</i>052	.185	.092
<i>Schizymenia</i>148	.416	.321
<i>Opuntiaella</i>202	.385	.314
Blank run before178	.182	.248

The columns in this table represent experiments run on different days. The blank run before indicates the amount of oxygen that was in the water to begin with. An increase in that amount, if it be in the blanks run at each depth, is due to the photosynthesis of diatoms, etc., in the water. A decrease conversely indicates that respiration has removed oxygen from the solution. On successive days sea water does not contain the same number of diatoms nor the same amount of oxygen. Therefore there will be some variation in the blanks.

It is evident from the results in table 1 and the first column in table 3 that a depth of 25 meters is too great for photosynthesis in any of the algae, while at 15 meters photosynthesis is still going on at quite a considerable rate in some of the algae. For this reason the lower basket was hung at a depth of 22.5 meters instead of 25 for the remainder of the experiments (columns 2 and 3). The results show that there is a slight positive photosynthesis at this depth in the red algae, but not in *Nereocystis*.

The results in the first column of table 3 were obtained at depths of 5, 15, and 25 meters. At a depth of 25 meters the results in the reds, *Opuntia* and *Schizymenia*, show very little difference from those of the water controls run at the same depth. In columns 2 and 3 the depth is 22.5 meters, and the results in these columns, using the same algae, show a very marked degree of photosynthesis. In no case, however, is there any evidence of synthesis in the brown algae at either 25 meters or 22.5 meters.

Polyneura and *Rhodymenia* were then tried, and the same results obtained (table 4), though they were not as reliable as those that were secured from more extensive experiments.

TABLE 4. *Ml. of oxygen produced by Nereocystis, a brown alga, and Polyneura and Rhodymenia, red algae, at different depths*

	6 a.m.—3 p.m. Clear, calm
5 meters	
Blank180
<i>Nereocystis</i>505
<i>Polyneura</i>400
<i>Rhodymenia</i>382
15 meters	
Blank169
<i>Nereocystis</i>296
<i>Polyneura</i>259
<i>Rhodymenia</i>185
22.5 meters	
Blank129
<i>Nereocystis</i>098
<i>Polyneura</i>222
<i>Rhodymenia</i>154
Blank run before129

TABLE 5. *Comparison of ml. of oxygen produced by Nereocystis, a brown, and Turnerella, a red alga, at different depths*

	6 a.m.—4 p.m. Calm, clear	6 a.m.—4 p.m. Calm, clear
1 meter		
Blank228	.233
<i>Nereocystis</i>935	.885
<i>Turnerella</i>867	.946
5 meters		
Blank228	.231
<i>Nereocystis</i>	1.031	1.031
<i>Turnerella</i>	1.047	.990
10 meters		
Blank225	.221
<i>Nereocystis</i>854	.905
<i>Turnerella</i>836	.870
Blank run before161	.158

In the experiments at shallower depths with *Turnerella* (table 5), it seems that the sunlight was too strong for maximum photosynthesis at a

depth of one meter. At a depth of 5 meters, photosynthesis is approximately at a maximum. There is little difference between a depth of one meter and 10 meters in the photosynthetic effect.

While in all cases more photosynthesis takes place in the algae at shallow depths, it is noticeable that the reds have the faculty of carrying on photosynthesis at a much greater rate than respiration, even as deep as 25 meters in Puget Sound waters. This probably accounts for the fact that reds are found deeper than browns and greens; that is, they are able to grow there, while at shallower depths, where they are not crowded out by the green and brown algae, I have observed them also. But very seldom have I found them in dense growths of other algae.

In tables 1, 3, 4, and 5 are to be found data which affect the light that

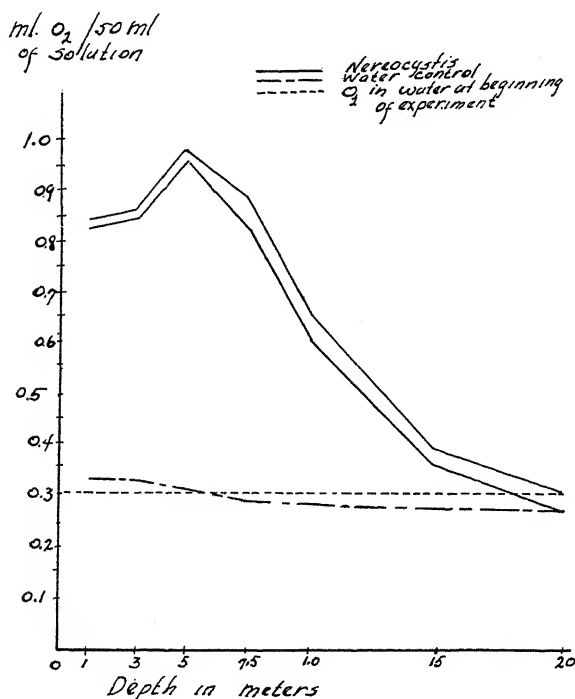


Fig. 1. Oxygen given off by strips of *Nereocystis* at different depths.

enters the water. Clouds, of course, decrease the amount of light entering the water, and choppy or rough water also decreases the amount entering. The most striking examples of this are to be found in columns 2 and 3 of table 3 and in figures 2 and 3. The experiment run in table 3 was from 6 a.m. to 3 p.m. on a clear, calm day. That run in column 3 was from 6 a.m. to 4 p.m. on a partly cloudy, choppy day. It will be noted that in the experiment run on the cloudy, choppy day, the photosynthesis at five meters was

much greater than that on the previous day at the same depth. It will also be noted that *Nereocystis* at 15 meters on the clear, calm day appears to have obtained enough light to carry on synthesis at a much greater rate than respiration.

The above experiments gave me an idea that there was maximum photosynthesis at about 5 meters' depth on sunny days.

Due to the lack of apparatus, I was unable to conduct experiments simultaneously at more than three different depths in the summers of 1931 and 1932. However, in 1933 I was able to obtain more baskets and so was able to conduct experiments at as many as eight different depths. This gave me sufficient data to plot photosynthesis at different depths with quite a fair

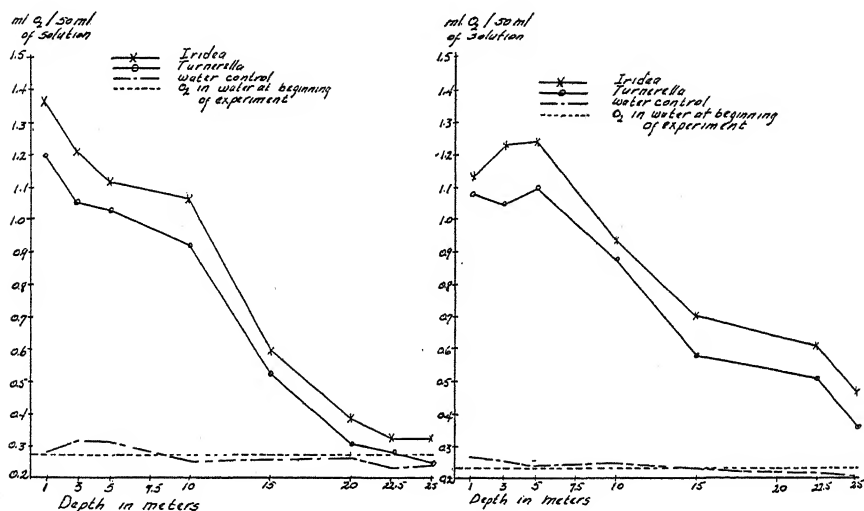


Fig. 2, 3. Fig. 2 (left). Oxygen given off by strips of *Turnerella* and *Iridaea* at different depths on a cloudy day when the water was slightly choppy. Fig. 3 (right). Oxygen given off by strips of *Turnerella* and *Iridaea* at different depths on a clear, calm day.

degree of accuracy (fig. 1). Figure 1 shows that at 1 and 3 meters photosynthesis in *Nereocystis* does not proceed at as rapid a rate as at 5 meters. From this maximum, photosynthesis gradually falls off until at 20 meters respiration equals or exceeds it.

Figures 2 and 3 show a comparison between photosynthesis of *Turnerella* and *Iridaea* on a cloudy day when the water was choppy, and on a clear calm day. Marked difference in the position of the photosynthetic maximum is evident. On the cloudy day the maximum was at the surface, and on the clear day the maximum was at 5 meters. It should be noted in this connection that in figure 2 active photosynthesis ceases at about 20 meters, due to lack of light, while in figure 3, on a clear calm day when maximum light may pene-

trate, photosynthesis is going on at a relatively rapid rate even as deep as 25 meters.

DISCUSSION

Though Moore, Whitley, and Webster agree with Engelmann that the red coloring matter, phycoerythrin, does not act as a color screen, but that it actually functions the same way that chlorophyll does in synthesis, it seems that there is no preponderance of evidence for this point of view. Ursprung (1917, 1918) projected the spectrum upon various green plants that were free from starch. The chlorophyll was then extracted and the leaves were tested with iodine for starch. It was shown that while there are regions in which greater carbon assimilation occurs, nevertheless there is some assimilation throughout the whole of the visible spectrum and in the ultra violet. Lubimenko (1923) has shown that different species of green plants are adapted to different light intensities. He states that it is only in the species adapted to a weak diffused light, such as *Hedera helix*, that the activity of the blue-violet rays becomes equal or superior to that of the red rays. He believes that the difference in the make-up of the individual plants enables those growing in diffused light to use the blue rays for the same work for which other plants use mostly red rays.

Shelford and Gail (1922), whose work is mentioned above, say that probably the color of the algae is complementary to the light at the sea depth at which they grow, because of the color of the light that penetrates to these depths. I am unable to account for their conclusions in the light of the fact that there are a great many of the red algae which are a bright red in color, yet grow at the surface or very near to it. In fact, there are some that are uncovered at low tide, and there are some browns, such as *Desmarestia* and *Agarum*, that grow in water from 10 to 15 meters deep.

It appears that more work needs to be done on the actual part that phycoerythrin plays in photosynthesis, before this question will be finally settled. It seems more probable that the red pigment acts as a color screen than as a photosynthetic pigment. It is very probable that the algae that grow in the depths are able to utilize the light of the shorter wave lengths. Most of the workers lay stress on the fact that in the absorption spectrum of chlorophyll the most intense absorption takes place in the region of the red rays, ignoring the fact that there is another band almost as intense in the blue-violet end of the spectrum (Willstätter and Stoll). There is a possibility that the red coloring matter may play a relatively unimportant part in synthesis in the red algae.

SUMMARY

1. Most photosynthesis takes place in both the red and brown algae in less than 10 meters of water.
2. It is clearly evident, however, that the red algae are able to utilize the light that penetrates to a depth of 22.5-25 meters, while the brown algae seem unable to synthesize carbohydrates in more than 15 meters of water.

3. Maximum photosynthesis takes place not at the surface, but usually at a depth of about 5 meters. This is probably due to the fact that the intensity of the light at the surface is too great for maximum synthesis. On cloudy days, or when the water is choppy, maximum photosynthesis is at the surface.

4. The view that the pigmentation of the red algae is correlated with the fact that they grow at a sea depth where the sun rays that penetrate are complementary to red—i.e., green—seems untenable in view of the fact that some red algae grow at the surface or very near to it, where the rays that penetrate are not complementary to red.

5. It is probable that the red algae are able to utilize the shorter wave lengths of light in much the same manner as do "shade plants." Most workers lay stress on the fact that in the absorption spectrum of chlorophyll the most intense absorption takes place in the region of the red rays. More attention should be directed to the absorption band in the blue-violet end of the spectrum.

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DO GERMINATING WILLOW CUTTINGS FIX ATMOSPHERIC NITROGEN?

C. A. LUDWIG

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It is well known that certain microorganisms growing alone and certain symbiotic pairs, each composed of a higher plant and a suitable microorganism, are able to assimilate the free nitrogen of the air. All other plants are commonly supposed not to have this ability. Nevertheless occasional suggestions, and even positive statements, are to be found in the literature to the effect that some of the ordinary higher green plants can utilize free nitrogen in comparatively large amounts, even independently of microorganisms. Work previous to 1924 has been reviewed by Lipman and Taylor (1924). Since then a number of additional suggestions of the same kind have been made, including one by Hicks (1928) that possibly germinating willow cuttings have this ability.

It is a matter of some theoretical and practical interest to determine whether such fixation does actually take place. The writer has therefore taken advantage of the ease with which willow cuttings can be germinated to investigate the change in nitrogen content which takes place during this activity.

Two experiments have been conducted. The first of these was carried out in 1929. Thirty-two 12.5-cm. cuttings, four each from eight different dormant shoots of *Salix* sp. collected near Arlington Farm, Virginia, were divided into four groups. Those of one group were dried at once for analysis, and those of each of the others were germinated, dried, and analyzed. All analyses were made simultaneously by the Kjeldahl method with CuSO_4 as the catalyst. Each group contained a cutting from each shoot, and the groups had equal numbers of basal, terminal, and intermediate cuttings, the purpose being to minimize the effect of any inequalities in the distribution of nitrogen through the shoot. The cuttings which were germinated were stood in distilled water in small beakers. Group 2 was placed under a bell jar in the laboratory with a 100-watt gas-filled Mazda lamp inside the jar to furnish light. Cooling was accomplished by standing the jar over water, lining it with wet filter paper, and allowing a stream of tap water to flow over the outside. Group 3 was placed under a second bell jar in the laboratory near the first but without any light source inside. Both bell jars were open at the top and were raised to allow air to enter at the bottom. Group 4 was placed on a window sill out-of-doors where it received full sunlight a little more than half the day

and skylight from about half the sky all day. Three of these last cuttings were lost. The experiment lasted from March 23 till April 27.

The results were calculated as percentages of the original green weight and are given in table 1.

The second experiment was conducted in 1932. Each shoot was cut into an uneven number of 15-cm. cuttings, which were numbered serially from the base of the shoot upward. Each odd-numbered cutting became a control and each even-numbered one a growth cutting. Analysis was by the Kjeldahl method with selenium as the catalyst. The amount of nitrogen originally present in each growth cutting was calculated by taking the mean percentage of the two adjacent controls. The apparent fixation was then determined in milligrams for each cutting, and the mean and probable error of the mean were calculated therefrom. To insure better growth than in the first experiment, the cuttings were stood in a 1/10 strength Burk's culture solution instead of distilled water and were placed under a source of artificial light which had been previously found suitable for plant growth (Ludwig, *in MS.*) As a result an increase of about 20 per cent in dry matter was secured as compared with none or a slight loss in the first experiment. The following is the composition of the nutrient solution as used: K_2HPO_4 , 0.08 g.; KH_2PO_4 , 0.02 g.; $MgSO_4 \cdot 7H_2O$, 0.02 g.; $NaCl$, 0.02 g.; $CaSO_4 \cdot 2H_2O$, 0.01 g.; $Fe_2(SO_4)_3 \cdot 9H_2O$, 0.001 g.; distilled water, 1 liter.

The cuttings were allowed to stand in one portion of medium for two or three days. They were then transferred to another and the first was boiled for several minutes to kill the microorganisms present. After two or three more days' time, allowed to permit the boiled solution to become reaerated, they were returned to the original solution, the second portion was boiled, and so on. The used solution was discarded and new supplied at occasional intervals. Blank cultures, without cuttings, were conducted to correct for traces of nitrogen which might be present in the distilled water or chemicals. All used solutions and all leaves, etc., shed from the plants were scrupulously preserved and included in the materials analyzed for nitrogen. Blanks and experimental cuttings were both dried in a vacuum desiccator over sulfuric acid and were analyzed simultaneously as in the first experiment in order to avoid systematic errors of analysis.

The cuttings were made from a series of shoots from a plant of *S. humilis* Marsh.,¹ found growing in Washington, D. C. They were cut, weighed, and put to germinate in part on each of the three days, March 7, 8, and 9. By March 18 the cuttings from the terminal portions of the shoots were all in full bloom, and the experiment was closed on May 6. At this time growth had ceased and many of the leaves were yellowish, as would be the case if nitrogen were deficient. The results are given in table 2.

¹ I am indebted to Dr. Alfred Rehder, Curator of the Herbarium, Arnold Arboretum, for the determination of this species.

TABLE 1.* *The apparent fixation of nitrogen by germinating willow cuttings in the first experiment*

Group	No. of cuttings	Nitrogen content (Percentage of green weight)	Apparent fixation (Percentage of green weight)
1	8	0.420 \pm 0.009	0.000
2	8	0.441 \pm 0.009	0.021 \pm 0.013
3	8	0.436 \pm 0.010	0.016 \pm 0.013
1†	5	0.438	0.000
4	5	0.417	— 0.021
2 + 3	16	0.438 \pm 0.007	0.018 \pm 0.011

* The nitrogen determinations were made by the late Mrs. Mary K. Murray, who at that time was a member of this laboratory.

† Results from the 5 cuttings which came from the same shoots as the 5 cuttings of group 4.

TABLE 2.* *Apparent fixation of nitrogen by germinating willow cuttings in the second experiment*

Cutting †	Original green weight (g.)	Calculated original dry weight (g.)	Total N originally (mg.)	Total N after growth (mg.)	Apparent fixation (mg.)
1-2	1.8825	.9977	12.4	12.8	0.4
1-4	1.2433	.6687	9.3	10.4	1.1
2-2	2.6481	1.4880	13.0	13.4	.4
2-4	1.9124	1.0571	11.0	11.5	.5
4-2	1.8993	1.0536	11.6	11.8	.2
4-4	1.4183	.7709	10.5	10.7	.2
5-2	3.9381	2.2634	17.5	18.5	1.0
5-4	2.9727	1.6613	15.4	16.3	.9
5-6	2.2484	1.2113	14.4	15.9	1.5
5-8	1.3202	.7035	9.7	10.5	.8
6-2	2.2316	1.2640	9.7	10.6	.9
6-4	1.7130	.9379	9.0	10.4	1.4
8-2	3.9905	2.2504	17.3	18.5	1.2
8-4	3.1138	1.7020	13.9	14.6	.7
8-6	2.6388	1.4652	13.2	13.8	.6
8-8	1.7777	.9800	10.7	10.8	.1
8-10	1.1244	.6013	8.1	7.7	— .4
9-2	4.6951	2.7185	20.5	20.5	.0
9-4	3.5077	1.9619	16.3	16.3	.0
9-6	2.7129	1.4620	14.3	14.7	.4
9-8	2.0341	1.0923	12.7	12.9	.2
9-10	1.1621	.6113	8.4	8.4	.0
Mean	2.3720	1.31465	12.68		0.55 \pm 0.063
Percentage of apparent fixation023	.042	4.34		

* Thanks are due for the determination of the nitrogen in these cuttings to Ellen K. Rist, of this laboratory.

† The first number in each case is the serial number of the shoot and the second is the serial number of the cutting, numbering from the base of the shoot upward.

Examination of these tables shows that the results from the two experiments agree very closely with each other and indicate that any fixation which

may have occurred is very small. The mean apparent fixation found in the 1929 experiment, using groups 2 and 3, is 0.018 ± 0.011 per cent of the green weight of the cuttings. The mean of the green weights of the cuttings in the two groups is 1.94 gm.; thus the apparent fixation amounts to 0.35 mg. per cutting. The size of the probable error indicates that this result is not significantly different from zero. If the fourth group were included, the result would be even lower; but it has been omitted because of its greater unreliability, due to the smaller number of cuttings involved, and because the apparent loss of nitrogen which these cuttings show can mostly be accounted for on the basis of the nitrogen gradient in the shoots between the checks and the experimental cuttings.

The mean apparent fixation per cutting found in 1932 is similarly low, 0.55 ± 0.063 mg. of nitrogen per cutting. In this case the small size of the probable error shows that the result is highly significant statistically—i.e., the uncertainty due to fluctuating variations has been eliminated and the result can be taken as indicating a definite slight fixation of nitrogen provided there is no source of systematic error which could alter the result without altering its probable error. In view, however, of possible errors in analysis, of the impossibility of maintaining completely sterile conditions, of other possible errors of unknown source, and of the fact that no correlation appears to exist between increase in nitrogen and the original size of the cuttings, it seems to be unjustifiable to conclude that any fixation of nitrogen has been demonstrated. It seems more reasonable to consider that the amount shown is well within the error inherent in the experiments. It should be noted that the increase is less than 5 per cent of the amount of nitrogen originally present. The smallness of the amount involved can be further appreciated by considering that even if it could be shown that no source of systematic error was present and that fixation unquestionably did occur the odds are 2621 to 1 that the amount of nitrogen fixed did not exceed 0.073 per cent (1.4 mg. per cutting) under the conditions of the first experiment and equally great that it did not exceed 0.87 mg. per cutting under the conditions of the second experiment. It seems reasonable, therefore, to consider the results as indicating that germinating willow cuttings of the species used and under the conditions provided do not fix free nitrogen, and as demonstrating that if they do the amount fixed is too small to be of significance in the metabolism of the plants.

This does not prove, of course, that non-symbiotic fixation of nitrogen by vascular plants does not occur. But it does make it important to examine carefully all other possible explanations to account for an observed apparent increase in nitrogen before deciding that such fixation has taken place.

SUMMARY

Two sets of willow cuttings have been germinated in the laboratory and nitrogen analyses made to determine whether or not any nitrogen was fixed. The results showed an apparent fixation of the order of one-half milligram

of nitrogen per cutting, a result which is believed to be within the error of the experiments. The results, therefore, are considered to indicate that no nitrogen fixation takes place under the conditions involved, and to show that in any case it is so small as to be of no significance in the metabolism of the plants.

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GEMMIPARY IN *BYRNESIA WEINBERGII*

HARRY N. STOUT

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Few plants have received more attention from investigators in so-called "regeneration" than *Bryophyllum calycinum* Salisb. The term "regeneration" has been used for the process by which new individuals arise from purely vegetative parts such as from leaf notches in *Bryophyllum*. This is the sense in which Loeb (1924) has used the term, making it a "different type" from that occurring in animals in which lost or injured parts are replaced. Much criticism has been directed against the use of this term as applied to plants, since with them the process is one of normal vegetative propagation (Reed, 1923) and not one of replacement of parts. More recently Yarbrough (1932) has offered objection to the use of the term. Although he has clearly pointed out its inapplicability, he has offered no other word as a substitute. It is in lieu of this that we here propose the term "gemmipary" for the phenomenon in question. The word has the advantage of having had current usage, and its application here does not materially broaden its meaning.

Members of the Crassulaceae exhibit gemmipary in varying degrees. *Bryophyllum*, with its numerous meristematic areas distributed along the leaf margin, is capable of producing many individuals from a single leaf, whereas other species with but a single meristematic area develop but one. In this latter class *Byrnesia Weinbergii* Rose belongs (fig. 1).

This species was chosen for study since leaves of it had been observed to produce young plants at their bases very soon after they had fallen off the stem upon the moist soil of a greenhouse bench (fig. 2). The origin and nature of the cells which initiate this new plant and their relationship to the other cells and tissues of the leaf suggested this inquiry. Only the histological aspect was studied and only this aspect is discussed here. Although my results do not parallel those which Naylor (1932) found in *Bryophyllum*, yet the similarities are so obvious that it is evident we are dealing with the same phenomenon. The greatest difference between our results lies not so much in the nature of the development of the new individual as in the stage of that development at which dormancy of the meristem occurs.

In a recent paper Naylor (1932) states that investigations on *Bryophyllum* have been directed along two distinct lines—viz., the physiological and the histological. The former has received the greater attention. He fully discusses the literature in so far as it deals with the histological phases of *Bryophyllum*. As the result of his research, he finds that the meristematic

areas present in the notches of a mature leaf are formed during the very early development of that leaf, while it is but a few millimeters in length, and do not originate from the phloem as stated by Beales (1923). These areas are distributed along the margin of the leaf separated by mature or maturing tissue. By the time the leaf is mature, these meristematic areas have developed to the stage of being "foliar embryos" which have leaf, stem, and root primordia and an absorbing "foot" which lies next to a vein. Normally, while the leaf is attached to the stem, the foliar embryos are in a dormant condition. Howe (1931) interprets these dormant structures as

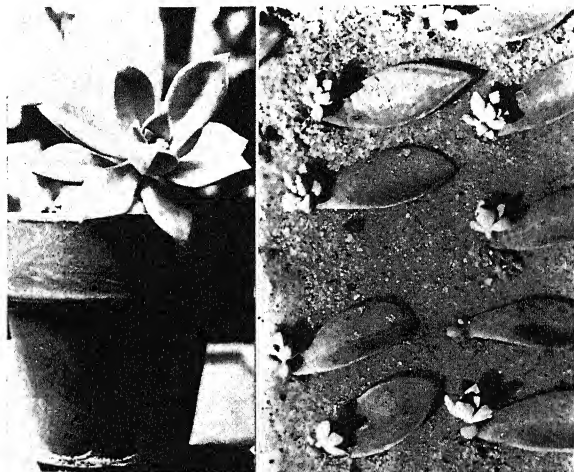


Fig. 1, 2. Fig. 1 (left). Young plant of *Byrnesia Weinbergii* Rose. Rosettes of leaves are formed at the ends of more or less prostrate branches. Courtesy of F. J. Hermann. Fig. 2 (right). Leaves of *Byrnesia* on moist soil developing new plants at the attachment end. Courtesy of F. J. Hermann.

buds, a term to which Naylor objects on the grounds that "recognizable primordia of the essential organs of the entire plant" are present. Hence an entire embryonic plant is indicated and not just a bud. Whether the term "bud" can be so limited in its application is open for questioning. About the second day after a leaf of *Bryophyllum* has been removed from a plant, growth is first evidenced by the multiplication of cells in the tips of the two root primordia. One root regularly grows a little in advance of the other as they make their way through the lower epidermis. The further growth of the whole embryo causes a thickening of the leaf in the region of the notch.

GROSS STUDY

Materials and methods

The material used in this study was taken from plants which had previously been grown from leaves secured from the University of Michigan

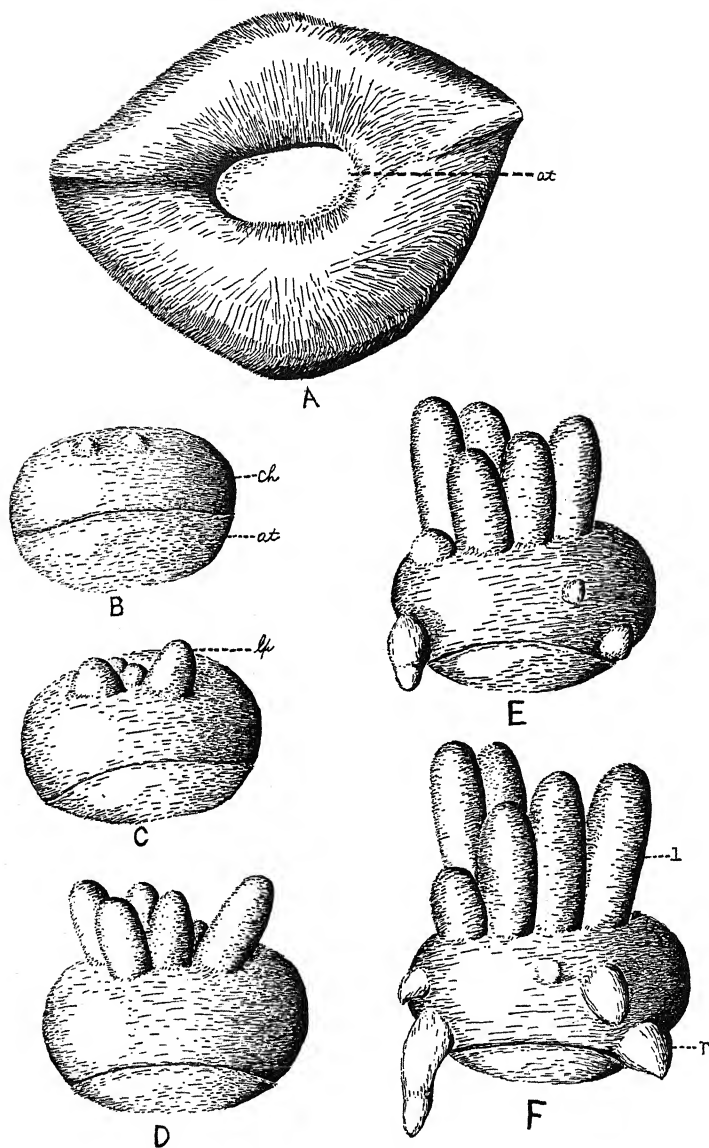


Fig. 3. *A*, drawing of basal end of leaf showing attachment area, *at*. *B-F*, different stages in the development of leaves and roots from the meristematic cushion located just above the attachment area; *lp*, leaf primordia; *l*, young leaves; *r*, root.

Botanical Garden through the kind permission of Prof. H. H. Bartlett. Only mature leaves from well-developed plants were used, although immature leaves proved equally capable of developing new plants.

Leaves of *Byrnesia* are very fleshy, approximating four or five centimeters in length, about one and one-half centimeters in width, and nearly one centimeter in thickness. In shape they are elliptical to broadly lanceolate, with a pointed apex and a slightly narrowed, sessile base. A mid-rib is somewhat evident on the upper surface, but all the lateral veins are too deeply imbedded to be seen. The underside of the leaf is slightly keeled. Although the base of the leaf is broad, averaging about six millimeters in width, it is actually attached to the stem by a very small elliptical area in the center. This ellipse is regularly 1.5 mm. in horizontal dimension and about 0.75 mm. in the vertical line. The elliptically shaped attachment area lies in a cup-like depression extending into the base of the leaf. There is a corresponding bulge on the stem over which the leaf is tightly appressed. Ridges are also apparent on either side of the scar which outlines the area of the stem indented by the leaf base. No axillary buds are visible either before the leaves are removed from the stem or for some time afterward, since they arise from meristems imbedded in the stem.

In order to observe all the external changes which take place during the development of a new plant at a leaf base, a dozen full-grown leaves were removed from well-developed plants and placed on moist sand on the greenhouse bench. The basal ends were examined from day to day with the aid of a binocular microscope, and a record of the observations was made by means of sketches (fig. 3, *A-F*).

Results

As has been pointed out, the abscission or attachment area occupies only a very small portion (fig. 3*A*, *at*) of the total area of the base of the leaf and lies in a depression. The first evidence of development consists in the formation of a small cushion of tissue located just above and back of the abscission area—i.e., adaxially to it. As this cushion (fig. 3*B*, *ch*) enlarges, it appears to push the upper margin of the abscission area outwards. In about ten days two small swellings (fig. 3*B*, *lp*) or protuberances situated close together appear on the upper surface of the cushion. These are the leaf rudiments which have the tip of the stem axis hidden between them. This is better shown in the microscopic section (fig. 14) to be discussed later. Almost daily, new leaf rudiments appear between the first pair (fig. 3*C*) until five or six of them are present in a close rosette. In about twenty-four days slightly smaller and more pointed protuberances (fig. 3*E*) appear, one on either side of the rosette of leaf rudiments and nearer the margin of the abscission layer. These are positively geotropic from the beginning, which identifies them as root rudiments. One root rudiment regularly appears a

little in advance of the other. Two or three similar rudiments appear later, at intervals, from the embryonic cushion. New leaf rudiments develop within the center of the rosette while the older ones increase in size and gradually take on the shape and character of mature leaves. The roots elongate until they make contact with the soil, at which time the young plantlet becomes partially independent, although apparently it is nourished by the parent leaf for some time thereafter. Long before the parent leaf has exhausted its food supply, the portion near the young plant becomes withered and shrunken until the two are finally separated.

HISTOLOGICAL STUDY

Methods

In order to study the histological changes and development which take place during the progress observed in the preceding discussion, a total of fifty full-grown leaves were removed from several plants and placed on moist sand in flats. These were covered with glass and placed on the greenhouse bench. At the same time four other similar leaves were prepared for immediate study by removing the basal ends about five millimeters from the place of attachment to the stem. Two of these bases were killed and fixed in chrom-acetic fixer and the other two in formalin-acetic-alcohol. Since the latter produced results the equal in every respect of the first and was more conveniently handled, it was used on all subsequent occasions. In dehydrating, Zirkle's (1930) butyl alcohol method gave results superior to ethyl alcohol and was therefore used for the greater part of the work. Sections were cut at ten microns and mounted serially. Cuts were made transversely through the leaf, longitudinally parallel to the leaf surface, and longitudinally tangentially to the leaf surface. Safranin counterstained with Delafield's haematoxylin or crystal violet was used to stain the sections. Both proved satisfactory for all general purposes. Drawings were accurately made with the aid of a projection apparatus.

Forty-eight hours after the leaves had been placed on the moist sand, four were removed and treated as described above. Similarly, at the end of each subsequent forty-eight-hour period, four leaves were removed and their bases imbedded until twelve such stages were secured. All the leaves used were in a healthy, firm condition.

Results

Histological studies made from mature leaves at the time they were removed from the stem show an area of meristematic tissue at the base of the leaf close to its place of attachment to the stem. Figure 4, which was drawn from a transverse section through this region, shows the degree to which this area is developed. It is well defined by the small size of the cells which change abruptly into the larger cells of the permanent tissues. This meri-

stematic or embryonic tissue is most abundant toward the upper side of the leaf directly above the vascular tissue, flanking it on either side and extending entirely around it as a narrow band underneath. The cells nearer the center of this area remain more active, since here cell division exceeds cell enlargement. The outer cells become differentiated and develop a cushion which, after pushing the face of the attachment area forward, becomes exposed above it. Tannin cells are more or less abundant both in and around the meristematic area, but regularly they extend in a well-defined band in the permanent tissue of the leaf immediately surrounding the meristem. This is brought out in figure 5, which shows a longitudinal section at right angles to the leaf surface through the leaf base. Numerous small bundles are present throughout the embryonic area with xylem elements predominating. Often the phloem is entirely lacking or consists entirely of parenchyma. Aside from this differentiation into vascular tissue, the embryonic area is homogeneous throughout. At this stage there is, then, no development of an embryo-like structure such as Naylor describes for mature leaves of *Bryophyllum*, but an undifferentiated area similar to the condition present in the very young leaves of *Bryophyllum* while they are less than a centimeter in length. No leaf or root initials can be recognized in the meristem of *Byrnesia*; neither is there a foot region acting as an absorbing organ, although the fact that the embryonic area is so sharply delimited from the permanent tissues of the leaf, together with the presence of many tannin cells, suggests the possibility that any portion of the meristem in contact with permanent leaf tissue may act in the capacity of an absorbing surface. *Byrnesia* stands in direct contrast to *Bryophyllum* in that, as the leaf develops from the primordium, a part of it near the base remains meristematic and becomes completely surrounded by permanent tissue. This meristem remains in a latent and undeveloped condition so long as the leaf is attached to the stem.

Development within the meristem begins within forty-eight hours after the removal of the leaf from the plant. Sections through the meristem at this interval (fig. 6, 7) show the beginning of this development. In figure 6, definite activity in the meristem is manifested by regions in which the cells are arranged in more or less concentric circles always centering about a group of xylem cells. In the longitudinal section (fig. 7) this early development is shown by a group of subepidermal cells near the upper surface which stain more intensely and initiate the protruding cushion illustrated in figure 3.

At the end of 96 hours, the concentric areas so noticeable at the forty-eight-hour stage are still apparent but less defined, resulting from the crowding of the cells through growth. At this time also, a sharply defined area of considerable extent in the central region of the meristem stains more deeply than the surrounding part (fig. 8, 9). The change in the size of the cells and in the density of the protoplasm is very abrupt from this inner meristematic region to the older meristem surrounding it and which was present as the dormant meristem. Already the developing meristem has

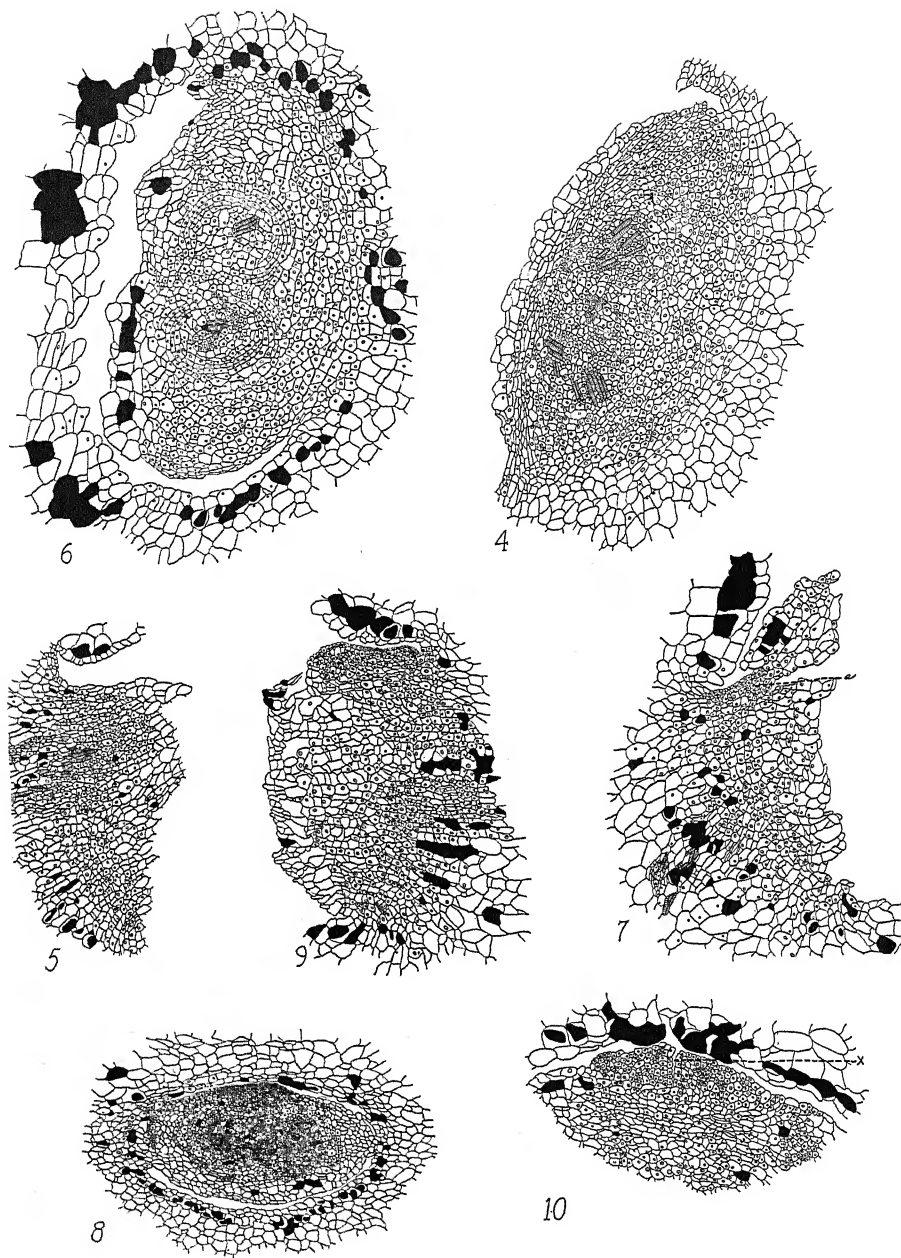


Fig. 4-10. Fig. 4. Transverse section through the meristem at the time the leaf is removed from the stem. Fig. 5. Longitudinal section through the meristem at the same time as fig. 4. It may be recognized by the small size of the cells. Tannin cells are filled in black. Fig. 6. Transverse section through meristem 48 hours after removal of leaf from the stem (explanation in text). Fig. 7. Longitudinal section through meristem at the end of 48 hours. Cells at *a* develop the cushion. Fig. 8. Cross section through the meristem at the end of 96 hours, showing its differentiation into inner and outer areas. Fig. 9. Longitudinal section through the meristem at the end of 96 hours. Abscission area is pushed forward and the cushion begins to protrude above. Fig. 10. Cross section through the meristem at the end of 144 hours. Lobes which become the first embryonic leaves lie on either side of the groove *x*.

pushed forward the abscission area and has begun to protrude above it (fig. 9, *a*).

Later stages in the development of the meristem show a continued increase in the amount of vascular tissue and in the size of the protruding cushion. At the end of 144 hours is found the first indication of an embryo-like structure (fig. 10, 11). A deep groove is formed by the greater growth of two initial regions on either side of a temporarily dormant one (fig. 10, *x*). These two active regions develop into lobes which become the first leaf primordia. The groove between them extends from the surface of the exposed cushion toward the interior of the meristem. At its base the cells form the tip of the stem axis (fig. 12) with its new leaf initials. This has attained considerable size at the end of 192 hours in our material. Longitudinal sections such as figure 11, which is slightly more advanced than the condition shown in figure 10, show that the innermost cells retain the maximum meristematic ability while those nearer the surface increase in size, causing the abscission zone to be pushed out farther and farther, while the meristem, growing above it, completes the development of the cushion.

The development of the root primordia seems always to lag behind the development of the leaf primordia and in our material was not seen until the end of the 240-hour interval. The roots are truly imbedded structures and mechanically force or dissolve their way through the surrounding parenchyma tissue (fig. 13). They have their origin at the outer margin of the inner meristematic area and usually occur in pairs, one a little in advance of the other. There is a break in front of the root tip in the meristem tissue as if the roots were lying in a pocket which seems to lengthen until the roots burst through to the outside. The root tips and leaf primordia do not lie in the same plane and at first are connected by the intervening meristematic tissue only. In all early stages development of root primordia is independent of that of leaf primordia. When the root begins to serve as an absorbing organ, a stelar connection is established between the root and stem axes.

By the time the meristem has grown for 288 hours, both stem and root primordia have attained considerable size, and are already visible on the exterior of the meristematic cushion (fig. 14). Further development of the embryo follows along the lines illustrated in figure 3.

STUDY OF AXILLARY BUDS

There is no external evidence of axillary buds either before or immediately after the removal of leaves from the stem. This suggested the possibility that the basal meristem might take the place of an axillary bud or that such a bud might have been removed at the time the leaf was taken from the stem. This was further suggested by the general absence of lateral branches except at the very base of the stem and then only a long time after the removal of the leaves. In order to determine the actual conditions present in a leaf axil, the following experiments were carried out. The lower leaves of a terminal

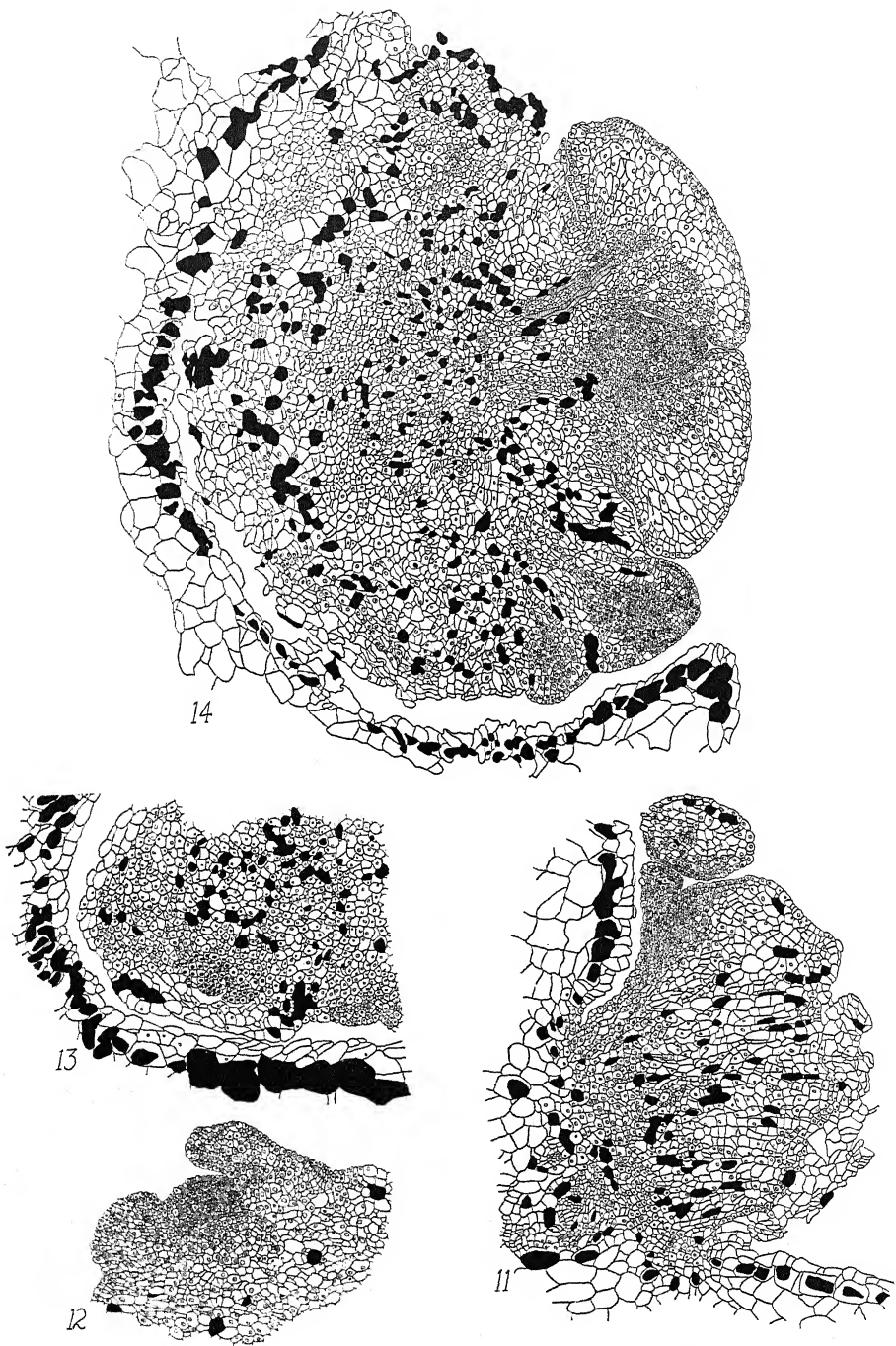


Fig. 11-14. Fig. 11. Longitudinal section through the meristem at the end of 144 hours, showing parts of the young plant. Fig. 12. Transverse section through part of the meristem, showing the young plant with stem tip and leaf primordia. 192 hours. Fig. 13. Transverse section through the meristem at the end of 240 hours showing the presence of root primordia. Fig. 14. Similar section after 288 hours (see text).

rosette were carefully removed from the stem and placed on moist sand. Each leaf scar was circled with India ink immediately upon removal of the leaf. The stem tip was then cut off in order to stimulate the production of lateral branches if possible. After a sufficient length of time, varying from two to three weeks, lateral branches appeared just above the leaf scars which had been encircled with ink. Also, the leaves which had been removed from these scars developed new plantlets at their bases. There is, then, no connection between the meristem which produces a new plant from a leaf base and that which produces a branch from a leaf axil. Sections of the stem cut through the node and kept serially revealed that the axillary meristem was deeply imbedded in the stem and that it lay at the base of a deep vertical groove. This meristem is likewise dormant so long as the leaf is attached to the stem. Its cells are undifferentiated into leaf or stem primordia, and aside from the lesser extent of the tissue and the absence of vascular elements, it is very similar to the meristem at the base of the leaf. Sections made through nodes at varying intervals of time after the removal of the leaves revealed that the development of the axillary meristem parallels that of the leaf meristem even to the production of root primordia.

DISCUSSION

The similarity between the foliar meristem and the imbedded axillary meristem raises the question as to what constitutes a bud as well as the hackneyed one of what constitutes an individual. The writer is inclined to support Howe's (1931) interpretation in calling the foliar meristems in the leaves of *Bryophyllum*, buds. The same must hold true for *Byrnesia*, since it has been pointed out that the difference in the meristems of the two plants is one of stage of dormancy and not of ultimate development. This development of dormant meristems has long been recognized as vegetative propagation or reproduction. Hence, the use of the term "gemmipary," reproduction by buds, for this phenomenon is justified.

SUMMARY

1. The tissue which initiates a new plant vegetatively in *Byrnesia* is a dormant meristem located at the base of the leaf. It surrounds the main vascular bundle, being broadest on the adaxial side. It is undifferentiated into root, stem, or leaf primordia, as has been described for similar meristems in *Bryophyllum*. No development of the meristem takes place while the leaf is attached to the plant.

2. The cells nearest the vascular elements divide the more rapidly, thus differentiating an inner and outer meristem. With the increase in the amount of the meristem, the abscission area is pushed outward, allowing the meristem to protrude above it.

3. Numerous vascular elements are formed throughout the meristem as it develops.

4. Leaf primordia are produced exogenously from the cells of the embryonic cushion. Root primordia are imbedded structures and reach the exterior by pushing through the meristematic area.

5. Differentiation of the meristem into a well-defined bud occurs within ten to twelve days after the leaf is removed from the stem.

6. Axillary meristems are imbedded in the stem at the base of minute slits and have no anatomical connection with the foliar meristems.

7. There are no anatomical differences between foliar and axillary meristems either in the resting stage or during the progress of their development.

Grateful acknowledgment is accorded by the author to Mr. Don M. Benedict of Temple University for suggesting the problem and for kindly directions and criticisms throughout its progress.

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ROCKY MOUNTAIN HERBARIUM STUDIES. II.

AVEN NELSON

(Received for publication May 1, 1933)

This paper is a continuation of the studies begun in the June number of the American Journal of Botany, volume 18, 1931. Critical examination of the available herbarium material in certain genera brings to light unnamed and misnamed sheets that cannot be incorporated in the known species. Other proposed species are based upon recent collections, by the writer and others, in various localities in the Southwest. It is hoped that a further paper, no. III, may follow within a few months.

Ephedra fasciculata Aven Nelson, new species

Stems rather slender, prostrate, only a few dm. long, the internodes 3-5 cm. long, 5 mm. or less in diameter, the older ones ash-gray; branches yellowish-green, numerous, closely fascicled, erect at right angles to the prostrate stems, short, having 3-5 nodes, the internodes 3-5 cm. long and 2 mm. or less in diameter, apparently smooth but under a lens obscurely striate and minutely roughened; nodes scarcely swollen, with two scarious scales apparently completely united into a close-fitting white truncate cup 2 mm. or less high, sometimes each scale showing a short rounded free tip; as the cup breaks down the base of the scales appears as a narrow brown band 0.5 mm. wide or less.

Flowers and fruit are not yet available, but the vegetative characters place it in the section with *E. viridis* Coville, and *E. antisiphilitica* Meyer, to neither one of which it seems possible to refer it.

It was secured in the hot dry banks of a sandy wash, in low hills near Phoenix, Arizona, May 1, 1925, by the writer. Collection number 10268; type in Rocky Mt. Herb.

Allium funiculosum Aven Nelson, new species

Bulb elongated, the bases of the two or more stems united by the common sheath which forms a tubular wrapping 5-10 cm. in length, the fibers coarse and abundant but not intricately interwoven; stems and leaves subequal, distinct as they emerge from the summit of the sheath, moderately stout, 3-4 dm. high, leaves flat, 2-5 mm. broad; involucre bracts 2, membranous, roseate; umbel erect, flowers 10-15, on short (6-12 mm.) slender pedicels; perianth deep-rose color, its segments oblong, acute 5-6 mm. long; stamens about equalling the flower segments; capsule globular, the crests small, or wanting.

The above description scarcely distinguishes this species from *A. Geyeri* Wats., Proc. Am. Acad. 14: 227. 1879. In fact, it probably should be considered as a subspecies of that, but its narrow elongated deep-set bulb with

the long coarse-fibred sheath (*funiculosum*—"full of cordage or coarse threads") makes it stand out from *A. Geyeri* in which the bulb is short and "onion" shaped with a closer network of smaller fibres. Geographically also they are well separated—*A. Geyeri* extending from Colorado into the Northwest. The specimens representing the proposed species are all from the Southwest. Castetter, 272 (type, in Rocky Mt. Herb.) and 964, co-type, Sandia Rim, 10500 ft., July 24, 1929; Goodding, 2426, Huachuca Mts., Arizona, Aug. 22, 1907; 179, Miller's Peak, Huachuca Mts., July 12, 1909; O. B. Metcalf, 716, Bear Mt. near Silver City, New Mex., Sept. 15, 1903; E. P. Walker, 357, Geyser Canyon, San Juan Co., Colo., July 30, 1902.

***Eriogonum umbellatum intectum* Aven Nelson, new variety**

Smaller than the species in every way, perhaps more surculose matted; only a little of the tomentum persisting even in early anthesis; later practically glabrous.

Doubtless many specimens of this species are extant, but the writer's no. 10695 may be cited as type, Rocky Mt. Herb. Secured in the low sandy hills of the northern part of the Red Desert, Sweetwater Co., Wyo., July 6, 1926.

***Purpusia Osterhoutii* Aven Nelson, new species**

Obscurely glandular-pubescent throughout: stems and leaves few—several, from the crown of the small tap-root, protected by remnants of former leaf-bases and stems; the pinnate leaves with 2–4 pairs of leaflets unequally spaced (the largest above); leaflets 5–10 mm. long, ovate, obovate or orbicular, more or less deeply cleft into obtuse teeth: petals yellow, minute, shorter than the ovate sepals. Receptacle contracted into a raised columnar base for the few achenes, only 1 or 2 of which mature.

This is an interesting find. The genus *Purpusia* described by Brandegee, Bot. Gaz. 27: 446. 1899, has stood until now as a monotypic one (*P. saxosa* Brand, l. c.). The species now added tallies closely with the original one but differs in the absence of viscosity and hirsuteness in the cymose inflorescence (in contrast to corymbose-racemose), the yellow petals (in contrast to white), the small crown with its few stems and leaves (in contrast to "caespitose"), and in the absence of hairs on the receptacle. Evidently the hypanthium also differs slightly in that the rim of the cup has a slight annular thickening from which the sepals, petals, and short filaments arise. (Cf. also N. A. Fl. 22³: 291. 1908.)

The type of this novelty is Mr. George E. Osterhout's no. 7103, Bright Angel Trail, Grand Canyon, Arizona, June 22, 1928. Again Mr. Osterhout has demonstrated his discriminating field work which so splendidly supports his occasional papers in the botanical journals.

***Astragalus jemensis* Aven Nelson, new species**

Caespitose on a branching woody crown, glabrate in appearance but, under a lens, showing a short white appressed pubescence throughout, the leaves

basal, on the crowns or very short stems, 1-2 dm. long; leaflets varying from 17-31, paired but usually not directly opposite, oblong to broadly oval or obovate, rounded at tip or somewhat retuse, 7-15 or even 20 mm. long; scapes 1 or more from each crown, surpassing the leaves, 2-3 dm. long, floriferous for one-half their length; flowers purple, several to many, in an open raceme, large, about 20 mm. long, the banner somewhat exceeding the other petals; calyx purplish, its tube about 10 mm. long, the subulate linear teeth 5-7 mm.; pod 1-celled, at first circular in cross-section, without intrusions, the walls somewhat thickened-fleshy, not at all inflated, becoming somewhat woody-coriaceous with the sutures conspicuous externally and with evident reticulations between; at maturity the pod is narrowly oblong, pointed, curved, and up to 3 cm. long.

Somewhat doubtfully this is being referred to the section *XYLOPHACOS*. In the Wooton-Standley Flora of New Mexico, its nearest ally is *Astragalus remulcus* Jones, Contr. 7: 658. This has much the same habit and technical pod characters, but there are no elements of confusion between them. Two sheets of Professor E. F. Castetter's no. 322, collected between Golden and Madrid (Jemez Mts.), N. M., May 23, 1930, are taken as the type (Rocky Mt. Herb.).

Galpinsia glandulifera Aven Nelson, new species

A short-lived perennial, only 1 dm. (more or less) high, blooming the first year and then with the aspect of a slender simple-stemmed annual with almost filiform root, the second year branching from the enlarging crown, the bases of these stems persisting, obscurely glandular on stems and leaves, less so on the calyx; leaves crowded especially upward, acute or obtuse at apex, usually tapering into a short petiole; calyx greenish-yellow, the tube 16-18 mm. long, expanding gradually into the obconical throat, the broad lobes, including the free tips 6-7 mm. long, petals saffron yellow, from broadly obovate to almost reniform, 1 cm. or more broad; young ovary fusiform, nearly half as long as the calyx-tube; mature capsule subcylindric, 8 mm. long, 2-3 mm. in diameter.

Type collection by Mrs. Gladys Convis, no. 37, on hills in vicinity of Carlsbad Caverns, New Mex., May, 1930. (Type: Rocky Mt. Herb.)

Chylisma arenaria Aven Nelson, new name

A large showy branching winter annual, or possibly more enduring. Grayish in appearance due to short white dense spreading pubescence especially on the earlier leaves and the main stems and branches, the later leaves and branchlets greener and less pubescent; leafy throughout, decreasing in size upward and passing into the bracts of the long fruiting raceme topped by the crowded flowers; leaves simple, dentate with numerous variable small sharp teeth, all broadly cordate and petioled, the larger with petioles 2 to 3 times as long as the blade, which is 2-3 cm. wide; calyx tube 1.5-2.5 cm. long, expanding gradually to the broad throat (5-7 mm.), its ovate-lanceolate lobes half as long; petals yellow (?) ageing pink-red, suborbicular, about 1 cm. broad; anthers large, pollen super-abundant; stigma large, a hemispheric disk; capsule linear-oblong, about 4 cm. long, usually sessile but sometimes tapering to a pedicel a few mm. long.

This proposed species is a segregate from *Chylisma cardiophylla* (Torr.) Small, Bull. Torr. Club 23: 193. 1896. It is Jepson's var. *longituba* of *Oenothera cardiophylla* (see Jepson's, Man. Pl. Calif. 686. 1925). It is also the *O. cardiophylla splendens* of Munz and Johnston. (See Bull. Torr. Club 49: 354. 1922.)

These specimens were secured by the writer, Feb. 26, 1930, in sandy washes in the Fortuna Range, some 20 mi. east of Yuma, Arizona, no. 11140 (type in Rocky Mt. Herb.). Since the locality is probably never visited by frosts, the time and length of the growing season are determined by the rain-fall. The herbaceous vegetation may start in the autumn and continue for several months, the length of time varying from year to year. The plants described were bushy-branched, some of them 6-8 dm. high.

***Euphorbia flagelliformis* (Engelm.) Aven Nelson, new combination**

E. Petaloidea flagelliformis Engelm., in Torr. U. S. & Mex. Bound. Bot. 185. 1859; *Chamaesyce flagelliformis* Rydb. Col. Agr. Exp. Sta. Bull. 100: 223. 1906.

***Dodonaea arizonica* Aven Nelson, new species**

A somewhat willowy branching shrub, 1 m. or more high, obscurely resinous-granular on the season's growth only: leaves glabrous, occasionally one or more having a varnished aspect but none adhesively viscous, narrowly oblanceolate or linear-oblong, mostly 4-6 cm. long but on vigorous vegetative shoots much longer, all acute, tapering at base to a short petiole: racemes short, subumbelliform, axillary or terminal on new shoots; flowers small, apetalous, calyx greenish, the 3 united styles thick, 6-8 mm. long, carpel-wings 6-8 mm. broad.

It will certainly result in clarity and understanding, and thus in service, if the *Dodonaea* of Arizona and adjacent Mexico be separated from that variable aggregate known as *Dodonaea viscosa* and first described by Jacquin in his Enumeration of the Plants of the Caribbean, in 1760. Since that time its known distribution has been extended to and through Mexico to Lower California. Its economic-medicinal and ornamental uses have caused it to be extensively grown in tropical regions.

The original species evidently occurred in the moist tropics, but in its wide dispersal other forms have arisen, most of which have not been ranked as species. As one would expect, where its range has extended into arid districts, with very different soil characters, the variations have been the most fundamental.

One such area occurs in south-central Arizona, and here we find a strongly marked *Dodonaea* that has from its earliest discovery borne the name of *D. viscosa*, var. *angustifolia*. In the judgment of the writer, this name is not tenable in case the name is raised to specific rank, as it most certainly should be. There can be only confusion resulting from retaining this plant under a trinomial name, especially when the trinomial originally must have desig-

nated something quite different. As remarked by Dr. Bailey, in his *Manual of Cultivated Plants*, 470. 1924, speaking of *D. viscosa*, "There seems to be confusion as to the limitation of this species, or it is exceedingly variable." For a fuller discussion and description, see, U. S. Nat. Herb. 23: 705. 1923 (Standley, "Trees and Shrubs of Mexico").

The original use of the name *angustifolia* was by Linnaeus, and certainly he did not refer to this willowy shrub of Arizona. The same must be true of Blanco, in his *Flora of the Philippines*, Ed. I: 312, as well as Thunberg, *Prod. Pl. Cap.* 77.

Furthermore, *D. arizonica* may well stand on its own merits in any case, for it has an aspect all its own, and the shreddy-fibrous gray bark contrasts sharply with the smooth dark-brown of the typical *D. viscosa*. The thinner narrow leaves are themselves diagnostic.

Representative collections of *D. viscosa* are Heller, 4507, Mayagues, Porto Rico, Feb. 3, 1900; Barkelew, 188, on Exped. to Revillagigedo Islands, Mexico, summer of 1903; Schaffner, 306, San Louis Potosi, Mexico, 1879.

Representative collections of *D. Arizonica* are Aven Nelson, 11276 (type in Rocky Mt. Herb.), dry stony hills, Salt River Valley, between Canyon Lake and Roosevelt Dam, Mar. 20, 1930; Jones, Mescal Mts., Ariz., May 24, 1890; Catalina Mts., Sabino Canyon, Aug. 18, 1903.

***Macrosiphonia dulcis* Aven Nelson, new species**

Low shrub, many-stemmed and more or less branched, from a woody branching caudex, 3–6 dm. high, the foliose branches slender and nearly herbaceous; leaves almost sessile, from narrowly to broadly ovate, entire and subacute or rarely obtuse and then broadly elliptic, somewhat puberulent especially beneath, 10–20 mm. long; flowers few, large, solitary, fragrant, singly in one or more of the uppermost pairs of leaves, sessile; calyx cleft to the pedicel-like tube which tapers slightly downward, tube and lobes subequal, each about 6 mm. long, the usual glands of the genus wholly wanting; corolla tube about 4 cm. long, the expanded portion of the throat somewhat longer than the distinctly puberulent slender basal part, limb of five obovate rounded spreading lobes, about 15 mm. long, white with (usually) roseate margins; anthers linear (not lance-subulate as in most other species), about 10 mm. long, subsessile at the base of the throat, subsagittate and tipped with a small triangular appendage, the basal part apparently not dehiscent; style slender, its large 5-costate stigma enclosed by the large approximated anthers but scarcely adnate; follicles not known; other characters resembling those of *M. brachysiphon* Gray, *Syn. Fl.* 2: 84.

Type in Rocky Mt. Herb.; L. N. Goodding's No. 2413, from the lower slopes of Miller Peak, Huachuca Mts., Mexico, Aug. 22, 1907.

***Gilia flavocincta* Aven Nelson, new species**

A small winter annual, 1–2 dm. high, related to *G. leptalea* (Gray) Greene, the slender stems simple at base but branching above into the open panicle inflorescence, blooming when only a few cm. high but becoming as much as 20 cm., or possibly more, the herbage green but at first with scattering obscure

lanate puberulence, in the inflorescence an equally obscure glandulosity; leaves few, those near the base crowded but not rosulate, linear, or pinnate with short linear pinnae, those on the upper stem sparser, smaller and usually entire; flowers large and showy for the size of the plant, blue but the expanded throat simulating a yellow girdle; calyx only 3-4 mm. long, green-ribbed with white puberulent membranes, the linear-cuspidate teeth shorter than the sub-campanulate tube; corolla tube slender, 6-8 mm. long, the throat about half as long and the rounded obovate lobes about 5 mm.; stamens inserted in the sinuses of the limb on filaments half as long as the anthers; seeds several, becoming mucilaginous in water and emitting spiracles.

Type: Rocky Mt. Herb., Aven Nelson, No. 11228, sandy soils, Apache Trail, near Canyon Lake, Arizona, March 20, 1930.

***Oreocarya Williamsii* Aven Nelson, new species**

Biennial or (?) perennial, densely setose-bristly throughout, the rosulate crowns borne singly or in small caespitose clumps; stems slender, simple, one only from each crown, suberect, 1-2 (or more) dm. high; crown-leaves numerous, linear-oblongate, 1-3 cm. long, canescent and sparingly setose-hispid, the earlier ones short, their bases persisting; stem-leaves narrowly oblongate and, above, linear; flowers crowded above with one or more small glomerules in the axils of the uppermost leaves: calyx-lobes subulate-linear, about 5 mm. long, equalling or longer than the corolla-tube; corolla pale-yellow but truly yellow throughout, not merely with a yellow eye; the tube proper very short (1 mm.), abruptly expanded into a semiglobular throat 2 mm. or more long; nearly closed by the conspicuous fornices, the limb 7-8 mm. in diameter, its lobes broadly obovate and entire; the included anthers on very short filaments standing at right angles to the wall of the expanded throat, thus holding the anthers free from the wall; style short, its stigma just below the anthers; nutlets (immature) apparently all maturing, ovate, at least one of them wing-margined and roughened on the back, the sides smooth.

The only species with which pubescence and the nutlets (in so far as these can be understood from the present material) permit this to be compared is *Oreocarya setosissima* (Gray) Greene. That, however, is a large coarse plant wholly different in aspect.

The points relied upon to distinguish this proposed species are its relatively weak suberect stems with greenish aspect, the absence of a canescent indument except on the older crown leaves, the numerous widely spreading setae, the yellow flowers, the narrow basal part of the tube of the corolla which is constricted above the ovary and then abruptly expanded into a nearly globular portion, within which the anthers stand on short transverse filaments.

Collected by Louis Williams in the Flaming Gorge, of Green River, Daggett Co., Utah; elevation about 6000 feet; June 2, 1932 (type No. 489 in Rocky Mt. Herb.).

***Penstemon regalis* Aven Nelson, new species**

Wholly glabrous and glandless throughout, subglaucous, the wand-like leafy simple stems (4-8 dm. high), terminating in a gorgeous narrow thyr-

soid raceme of regal crimson-purple flowers; leaves entire, thick, the basal oblanceolate to obovate, the lowest ones on petioles as long as the blade, becoming longer, broader and sessile as they pass into the cauline, mid-stem leaves broadly ovate, up to 6 cm. in length, with the aspect of cordate-perfoliate but completely distinct as are the greatly reduced bracts of the inflorescence; calyx short, the sepals ovate-lanceolate, distinct nearly to the base, 4-5 mm. long; corolla 20 mm. or more long, tubular, expanding upward past the middle and then tapering slightly into the narrow throat, both lips very short, the oval-ovate lobes only 3-4 mm. long, inordinately bearded on the lower lip with long yellow hairs; sterile filament slightly flattened and bearing a few to several hairs below the tip; anthers in the throat, the filaments free except for the lower one-third which is superficially attached to the very base of the tube; anthers somewhat explanate, opening the full length but not through the junction; style extending into the beard of the lip.

This beautiful *Penstemon* was secured near the Carlsbad Caverns of New Mexico, by Mrs. Gladys Convis, in May, 1930. Her number 75 is the type, in Rocky Mt. Herb.

***Amphipappus spinosa* Aven Nelson, new species**

A low intricately branched shrub, 2-3 dm. or more high; the old stems with gray shreddy bark; branches nearly white, with thin glabrous cracked bark, the twigs slender, pale green with an obscure puberulence, many of them naked and distinctly spine-like: leaves numerous, chiefly on the younger branchlets, the puberulence similar to that of the branchlets, green, oblong or oblanceolate, obtuse or acutish, short-petioled or subsessile: heads few, solitary or in small clusters, about 5 mm. high; involucre pale, glabrous, the few thin outer bracts broadly oblong, obtuse, the inner longer and narrower and somewhat scarious and fimbriate at tip: ray flowers 1 only, its ligule oblong, with 3 minute teeth at its truncate tip; the achene finely pubescent and the few pappus scales fimbriately cut; disk flowers 3-5, normal in appearance but probably sterile, the 8 or 10 bristles as long as the corolla, variously kinked or tortuous.

Collected by L. N. Goodding, no. 707, on the Virgin River, in southern Nevada, May 5, 1902. Type in Rocky Mt. Herb.

This species is as remarkable as the type of the genus, *Amphipappus Fremontii* T. & G., and follows the original in generic characters very closely. The intricate branching and the spinescent character of so many branchlets serve for ready separation. The puberulence will also distinguish it from the original.

To follow recent precedent, one should call this *Amphiachyris spinosus*, but I doubt the advisability of merging *Amphipappus* T. & G. and *Amphiachyris* Nutt. The latter, while antedating the former, was based upon an annual herb, *A. dracunculoides*, differing in many respects from the desert shrub of Torrey and Gray. The wholesale segregation of genera is to be deplored. A genus should be recognizable in the field. If a scalpel and lens are required to detect the diagnostic characters, the splitting has gone too far. On the other hand, if two species in a given genus must be dissected before

relationship can be established, the segregation has not gone far enough. No field-worker, I am sure, finding *Amphiachyris dracunculoides* and *Amphipappus Fremontii*, would even suspect their relationship, close as it is. For this reason it seems logical to retain *Amphipappus* as originally characterized. It is a striking genus.

***Machaeranthera hiemalis* Aven Nelson, new species**

A pale ashy-green winter annual, 3-4 dm. high, the single axis branching more or less corymbosely from near the base upward, the entire plant with a thin pale puberulence: leaves firm, undulately toothed to entire; the lower petioled, becoming smaller, sessile, somewhat clasping, and finally bract-like, varying from oblanceolate through oval-oblong to narrower, mostly obtuse: heads small to medium, the involucre 6-8 mm. high and 10 mm. broad (more or less); bracts linear-oblong, the outer obtuse, the inner acute, pale with green tip, some finally reflexed, minutely glandular in the puberulence, as are also the peduncles and branchlets; rays short, pale-pink; pappus fuscous, the achenes short, obscurely pubescent.

In duration and habit it suggests *M. tanacetifolia* (H. B. K.) Nees., but in its long slender vertical tap-root it is unique. One wonders just what was included in *M. montana* Greene (Pitt. 3: 60), "High Plains of Wyoming to the Eastern slope of the high Californian Sierra." In Pitt. 4: 22-24, Dr. Greene removes from the original aggregate *M. pulverulenta*, *divaricata*, and *viscosa*. "The name may now stand for the plant of the Californian Sierra," and then he re-defines the species but names no type. Were it not for the mountain habitat, the pinnate leaves, and the duration ("Perennial") of his *M. montana*, one might suspect that *M. hiemalis* should be referred to it.

Collected by the writer (no. 11190, type in Rocky Mt. Herb.) in Devil's Canyon, near Jacumba, Calif., Mar. 14, 1930. This locality is on the western border of the Imperial Valley where the canyon temperature is high even in the winter, especially in the volcanic sands. The tap-root and foliage indicate that the life-history of the plant is completed in one growing period, which in this case consists of the calendar winter months.

***Erigeron lobatus* Aven Nelson, new species**

A large handsome winter annual 5-8 dm. high, mostly branched throughout, sometimes with one or more accessory stems from the base: pubescence hirsute throughout, spreading, sparse: leaves abundant, crowded on the crown, gradually more open and smaller upward, becoming bract-like on the slender peduncles; the lower leaves 8-15 cm. long including the narrowly margined petioles, spatulate-oblanceolate in outline, deeply pinnately lobed, the broad lobes mostly obtuse, upward tending to become entire and finally linear-oblanceolate: peduncles 5-10 cm. long, naked or sparsely foliar-bracteate: heads medium-large, the involucre hirsute, especially at base, the bracts green, linear, 3-4 mm. long; rays numerous, 2-3 times as long as the bracts, violet-blue; disk flowers very numerous, the achenes glabrate.

The species proposed is clearly of the *Erigeron divergens* series. Taking

the extremes of this series, one could not desire more clearly defined species. Because of the intergrades one hesitates to add another name, but a form so characteristically developed as *E. lobatus* is interesting enough to carry its own cognomen.

From the writer's collection in the Salt River Canyon, Arizona, on the Apache Trail, near Canyon Lake, no. 11209, Mar. 19, 1930. Type, Rocky Mt. Herb.

***Arnica Maguirei* Aven Nelson, new species**

Plant strictly erect, 1 meter, more or less, high; the unbranched stem slender for its height, glabrate below but becoming minutely granuliferous and sparsely lanately-pubescent upward: leaves entire, large, 7-10 pairs equally distributed, the basal narrow, with long margined petioles connate into ocreae 2-4 cm. long, these withering early but persisting for a time; stem-leaves elliptic-oblong, rounding into the broad margined petiole which with the sheath shortens and disappears entirely in the uppermost sessile pairs; leaf-blades 12-20 cm. long, 4-7 cm. broad, conspicuously veined; the uppermost smaller, becoming acute and the floral pair acuminate, glabrate in appearance but with a sparse soft pubescence: heads 3 or more (the central one and a pair out of the uppermost foliar-bracts or, if more, an additional pair from the next lower bracts) on erect naked peduncles 8-15 cm. long; involucre bracts linear-oblong, obtusish, less than 1 cm. long, lightly pubescent: rays medium size but conspicuous, well surpassing the darker disk; disk corollas softly pubescent, equalled by the sparse tawny minutely barbellate pappus; achenes brown, lightly striate and nearly glabrous.

This extraordinarily tall *Arnica* with its simple wand-like stem carrying its numerous large thin leaves has an aspect all its own. Perhaps typical *A. subplumosa* Greene might come to mind but the smaller plant and much larger heads of that as well as its notable pubescence and glandulosity exclude it from consideration.

This species was secured by Prof. Bassett Maguire of the Agricultural College of Utah, at Logan. At my request he has supplied the information which follows: "I remember distinctly the exact station, for at the time I was particularly impressed with the great size of all the plants. A few specimens only were taken from a considerable colony but those collected are wholly representative. The station lies in a low, grassy open woodland of willow and cottonwood, north of the outlet of Lower St. Mary Lake, Alt. 4460, Glacier National Park, Aug. 4, 1932." Number 1098, in the Rocky Mt. Herb.

Since Mrs. Maguire (Ruth R.) is also a botanist and joins her husband in his field and herbarium studies, I am giving myself the pleasure of dedicating to both of them this fine species, *Arnica Maguirei*. Their collection number is 1098. The type is deposited in the Rocky Mt. Herb.; co-types at Utah State Herbarium, Logan, and in the Cornell Herbarium.

***Arnica trina* Aven Nelson, new species**

Plant 4-5 dm. high, glabrate in appearance though with scattering crisped hairs throughout and some glandulosity on the peduncles and especially at the

base of the involucre: stem stoutish, with large equably distributed leaves; the basal pair (or pairs) small, sessile, spatulate-oblong, 2-3 cm.; the lower cauline pair broadly obovate, narrowed to a very short winged petiole, conspicuously serrate-toothed, 6 cm. or more long, 4 cm. or more broad; venation characteristic—consisting of a strong mid-vein, a pair curving from the base and uniting at the apex and a second lighter pair paralleling the first; the second and third cauline pairs similar but larger, elliptic-oblong and tending to become acute at apex; the floral pair scarcely smaller, sessile by broad rounded base, narrowly triangular-lanceolate; inflorescence bi-ternate and normally consisting of 9 conspicuous heads corymbosely arranged, the three primary rays about equal, 6-8 cm. long, and again ternate from a pair of foliar bracts on each, the three sets of secondary rays subequal, 3-4 cm. long; involucre small for the large golden-yellow rays, dark sordid-green, mostly less than 1 cm. broad and about as high, the fully expanded head 3-4 cm. broad; the disk dark by reason of the well exserted stamens and the still longer style; the short pappus tawny and subplumose; achenes dark-brown and with scattering stiffish hairs.

This beautiful *Arnica* was also secured by Prof. and Mrs. Maguire in Glacier Park. It was found in the margin of woods overlooking Lake Josephine at an altitude of about 4880 ft., Aug. 2, 1932. In the field it was mistaken for *A. diversifolia* Greene, to which indeed it is most closely allied. Examination of Greene's type number from Oregon shows that species to be much smaller, the leaves definitely petioled and fewer, less coarsely and saliently toothed, the inflorescence consisting of 1-3 heads from the floral pair of leaves, the involucre much larger and broader while the rays are fewer and shorter. The involucre in *A. trina* is definitely campanulate and not turbinate as in *A. diversifolia* and in many other species. The leaves differ markedly—those of *A. trina* being a deep green with a definite sheen.

The type bears the number 1095 and is deposited in the Rocky Mt. Herb. Co-types in Utah State Herb., Cornell Herb., U. S. Nat. Herb., and Gray Herb. A second collection of it by the Maguires, no. 1094, shows all of the essential characters, though the inflorescence is not so fully and definitely bi-ternate. This number was secured in a similar situation at Elrod Lake and is named with the type number.

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THE EFFECT OF CERTAIN CHEMICALS ON THE CATALASE ACTIVITY IN PLANTS¹

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The catalase activity of plant tissues fluctuates with changes in their metabolic activity or physiological condition. The measurement of these fluctuations has been used as a means of ascertaining the response of plants to various treatments. Heinicke (1928) states that despite the fact that nothing definite is known as to the metabolic rôle of catalase, the measurement of its activity is a reliable and sensitive index to changes occurring in the internal condition of plants.

The studies reported in this paper were made for the purpose of determining the effects of several chemicals of known herbicidal value, and some related compounds, on the catalase activity of living plants and plant-tissue preparations. The information is of interest, as there is little mention in the literature concerning the effect of the chemicals studied on catalase activity. Some improvements in the technique of making catalase determinations are also described.

EXPERIMENTAL

Materials. As the investigation was conducted during the winter months, it was necessary to work with a species of plant adapted to indoor cultivation. Cabbage was used, as it grows well in the greenhouse and has leaves of such size that sampling causes a minimum of shock and injury to the plant. The plants, Copenhagen Market variety, were grown in six-inch pots from one well-mixed lot of seed and were selected for uniformity in age, size, and apparent vigor.

Watering was controlled by giving each plant 150 cc. of tap water at about the same time each day. Plants selected and handled as described were found to have approximately the same initial catalase activity.

Cabbage leaf tissue is fairly homogeneous as to catalase activity. Samples collected 24 hours apart from 25 untreated plants over a period of several weeks showed a maximum difference in catalase activity of five per cent.

Chemicals. The chemicals used were in aqueous solutions of 0.01, 0.1, and 1.0 molar concentration. The ammonium sulfocyanate, ethylene oxide, diethylene oxide, propylene oxide, and sodium chlorate were of technical grade, while the thiourea and sodium arsenite were of C.P. quality. Some

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preliminary work did not show any appreciable difference in the effects of technical grade and C.P. chemicals on catalase activity.

Potted plants were treated by substituting 150 cc. of chemical solution for the daily allowance of water. Tissue preparations were treated by pipetting 1 cc. of chemical solution into the arm of the reaction tube containing the tissue preparation aliquot about a minute before mixing. Since some of the chemicals reacted slightly with hydrogen peroxide, blanks were run and corrections made where necessary.

Samples. Each plant was sampled twice. The first sample was taken from the right side of several leaves of a plant immediately before treatment, while the second sample was cut from the left side of the leaves previously sampled 24 hours after treatment. Ten discs of leaf tissue, cut by means of a sharp one-cm. cork borer pressed down upon a rubber stopper, and weighing from 200 to 230 mg., comprised a sample.

Certain of the treatments caused severe wilting. When this occurred, the second sample was prepared as if its weight were the same as that of the first sample. In the absence of wilting, the two samples seldom differed in weight by more than a few milligrams.

Tissue preparations. A sample was prepared for testing by grinding it for two minutes in a small mortar together with an equal weight of precipitated chalk, a pinch of acid-washed quartz sand, and one-half cc. of distilled water. A large pestle facilitated grinding. The thin paste resulting from the grinding was then washed into a small beaker with sufficient water to make a final dilution of one part of tissue to 50 parts of water.

The difficulty of drawing comparable aliquots from catalase preparations has been commented on by Heinicke (1924), Knott (1927), Overholser (1928), Leggatt (1929), and others. All errors from this source were eliminated by mechanically agitating preparations previous to, and during, the drawing of aliquots. A mechanical agitator was made by clamping a small battery motor to a ringstand in a vertical position. A glass rod flattened at one end and attached to the motor shaft by means of a short piece of heavy rubber tubing served as the stirrer. A perforated rubber stopper held in a burette clamp was used as a bearing for the stirring rod. The speed of the motor was regulated with a rheostat so that frothing of the preparation was prevented.

Hydrogen peroxide. The strength of the hydrogen peroxide was adjusted by dilution with distilled water so that 1 cc. would evolve exactly 12 cc. of oxygen when mixed with one-half gram of manganese dioxide. Crocker and Harrington (1918) state that all that need be known about the hydrogen peroxide used in catalase determinations is its strength, even though it contains an acid preservative. It was not considered necessary to neutralize the hydrogen peroxide, since the tissue preparations were well buffered by the excess of precipitated chalk they contained.

The mixing apparatus. The essential features of the mixing apparatus

are shown in the photograph. It is well known that the activity of most catalase preparations made from plant tissues declines more or less rapidly from the time of preparation. As the rate of decline usually becomes less rapid with the lapse of time, many workers have allowed their preparations to stand from one to 24 hours before testing. Heinicke (1923) found that the results

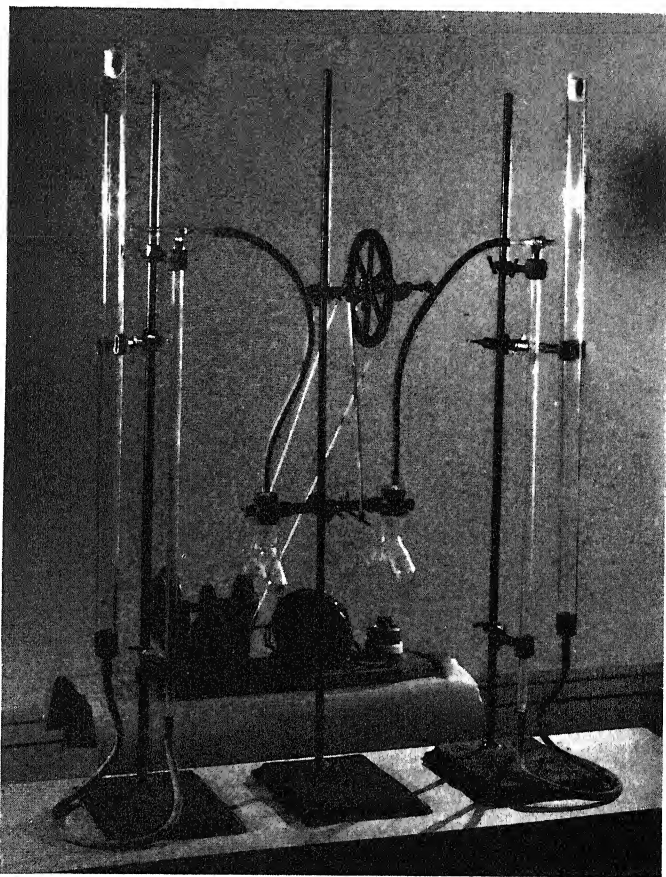


Fig. 1. Photograph of mixing apparatus with reaction tubes in place. A water bath held at 25°C., in which the tubes are immersed, is not shown.

of consecutive tests on fresh preparations seldom agreed. By testing aliquots simultaneously, almost perfect check results were obtained without a period of waiting. It also seems that the true catalase activity of tissues is more likely to be approximated if as little time as possible is allowed to elapse between preparation and testing. The average time required to make a duplicate determination, including sampling, was about 20 minutes. Harding (1930) used a duplex apparatus of different design with good results.

The slow reciprocating motion imparted to the reaction tubes is preferable to violent shaking, as there is less chance of the hydrogen peroxide spontaneously breaking down. The tube contents were mixed about 44 times per minute. Heinicke (1923) found a moderate rate of mixing as efficient as one that was quite rapid.

The reaction tubes were immersed in a water bath, not shown in the photograph, that was held at 25°C. Blank runs showed no evolution of oxygen at this temperature.

The reaction tube holders were made by attaching large hose clamps to short lengths of pipe. The pipe was of such bore that it fitted over the rocker shaft. Slots cut in the open ends of the pipes that engaged pegs set in the rocker shaft held the reaction tube holders in a fixed position. Should readings taken every minute be desired, one tube holder can be held away from its peg until mixing has taken place for several seconds in the other tube.

RESULTS

Whenever considering the effect of chemical or other treatments on catalase activity, it may be well to bear in mind the statement of Morgulis (1921), who is of the opinion that little credence can be given to the quantitative results of catalase experiments unless large differences are demonstrated.

It will be noted that there was generally more or less difference in the extent to which catalase activity was affected by similar treatments. This may have been due to fluctuations in certain environmental factors, such as transpiration rate. Stewart and Smith (1922), however, think that the specific nature of a plant is an important factor in its behavior toward poisons. Lyon (1917) commented on the differential resistance of plants from similar lots to arsenical poisoning. An early worker, Chatin (1845), stated that the effect of arsenic on plants is influenced more by the constitution of the individual plants than by their age. Despite differences in the magnitude of the results, the trends were in the same direction.

None of the chemicals used induced an increase in the catalase activity of tissue preparations. This is in accord with the work of Denny, Miller, and Guthrie (1930), who were unable to find a chemical that would accelerate the catalase activity of juice expressed from potato tubers. These workers noted that certain chemicals which had little or no effect when present in weak concentration would pronouncedly depress activity when added in quantity.

The results of the treatment of four plants and two tissue preparations are given for each concentration of the chemicals used. Only two tissue preparation treatments were included, since they agreed much more closely than did those of the plants. The trends of all treatments were verified by a number of experiments, the results of which are not presented.

The volume of oxygen evolved from 2 cc. of hydrogen peroxide by 2 cc. of tissue preparation at the end of 10 minutes' mixing was taken as the measure of relative catalase activity. The data presented show the percentage of

change in catalase activity that occurred in plants 24 hours after treatment and in tissue preparations immediately after treatment. They are based on averaged duplicate determinations that checked within one per cent. A minus sign before a figure denotes a decline in catalase activity.

Ammonium sulfocyanate. NH_4CNS . The results of the treatment of potted plants were as follows: (.01 M) 0.0, 1.9, 3.6, and — 4.5 per cent; (.1 M) — 3.4, — 6.4, — 10.0, and — 10.3 per cent; (1.0 M) — 70.7, — 85.0, — 86.6, and — 95.7 per cent. Some of the plants given the .01 M treatment showed slight increases in catalase activity. This was also true in the case of sodium chlorate. These changes were not considered significant, since they were less than those shown by untreated check plants.

Tests were made for the presence of ammonium sulfocyanate by means of ferric chloride, which forms a bright red precipitate with the sulfocyanate. None of the plants receiving the .01 M treatment contained appreciable amounts of ammonium sulfocyanate. These plants appeared to be uninjured. Of the plants given the .1 M treatment, the two showing the greatest decreases in activity had ammonium sulfocyanate in their vascular tracts and were slightly wilted. All of the plants treated with the 1.0 M concentration contained sulfocyanate in quantity. These plants were badly wilted and had a bleached appearance. From this it seems that there was a direct correlation between reduction in catalase activity and the amount of ammonium sulfocyanate present.

The results of the tissue preparation treatments were as follows: (.01 M) — 10.0 and — 11.8 per cent; (.1 M) — 60.8 and — 67.5 per cent; (1.0 M) — 78.7 and — 80.4 per cent.

According to the classification of Harvey (1931), ammonium sulfocyanate may be considered a protoplasmic poison. This worker states that protoplasmic poisons check the activity of certain enzymes, coagulate proteins, or combine with some of the cell constituents. The affinity of ammonium sulfocyanate for iron may, in part at least, account for its toxicity.

Thiourea. $(\text{NH}_4)_2\text{CS}$. All concentrations of this isomer of ammonium sulfocyanate reduced catalase activity considerably. The results of the treatment of potted plants were as follows: (.01 M) — 18.2, — 18.7, — 26.8, and — 37.9 per cent; (.1 M) — 47.2, — 66.7, — 69.0, and — 80.3 per cent; (1.0 M) — 62.0, — 78.5, — 82.8, and — 91.5 per cent.

Despite the pronounced decreases in catalase activity, the plants treated with thiourea had a much better appearance than those to which ammonium sulfocyanate was applied. The only evidence of injury was a slight wilting of the plants given the 1.0 M treatment. Thiourea also reacts with ferric chloride, though the precipitate formed is not as distinctively colored as in the case of ammonium sulfocyanate. A positive test was noted in all plants given the .1 and 1.0 M treatments.

Taking all concentrations used into consideration, thiourea was less effective in reducing the catalase activity of tissue preparations than ammonium

sulfocyanate. The results of the addition of thiourea to tissue preparations were as follows: (.01 M) — 9.2 and — 13.0 per cent; (.1 M) — 18.4 and — 21.7 per cent; (1.0 M) — 35.6 and — 40.2 per cent.

The toxic action of thiourea may be similar to that of ammonium sulfocyanate.

Ethylene oxide. C_2H_4O . All treatments with this chemical caused increases in the catalase activity of potted plants. The results of the treatments were as follows: (.01 M) 8.2, 14.9, 32.2, and 43.3 per cent; (.1 M) 9.0, 21.7, 23.8, and 66.7 per cent; (1.0 M) 24.4, 25.0, 26.1, and 33.7 per cent. The only evidence of injury was a slight wilting of the plants receiving the 1.0 M treatment. Many of the lower leaves of the treated plants showed a purplish coloration. This has also been noted by Harvey (1931).

The stimulating effect of ethylene compounds on catalase activity has been noted by several workers. Guthrie (1931) found that ethylene chlorhydrin accelerated the catalase activity of potato tubers. Nord and Franke (1928) reported that treatment with ethylene gas increased the catalase activity of tobacco plants as much as 20 per cent.

The results of the tissue preparation treatments were as follows: (0.1 M) 0.0 and — 0.9 per cent; (.1 M) — 2.1 and — 3.3 per cent; (1.0 M) — 21.3 and — 25.6 per cent.

The toxicity of ethylene oxide is somewhat different from that of the characteristic protoplasmic poisons. It may increase permeability, or in some way accelerate certain of the vital processes, since ethylene treatments have been found to increase the rate of respiration.

Diethylene oxide (dioxan). $C_4H_8O_2$. This polymer of ethylene oxide was found to depress the catalase activity of potted plants. The results of the treatments were as follows: (.01 M) — 5.6, — 7.0, — 8.1, and — 8.1 per cent; (.1 M) — 7.6, — 8.6, — 16.1, and — 16.3 per cent; (1.0 M) — 11.1, — 15.4, — 25.6, and — 34.1 per cent. The interesting feature of these treatments was that none of the plants appeared to have suffered injury, as they were all in apparently healthy condition several days after treatment. Harvey (1931) found that diethylene oxide was not toxic to plants when applied in doses equal to those of ethylene oxide that were lethal.

The results of the treatment of tissue preparations were as follows: (.01 M) — 4.4 and — 5.1 per cent; (.1 M) — 10.3 and — 13.2 per cent; (1.0 M) — 30.8 and — 35.9 per cent.

Propylene oxide. $CH_3(CHCH_2)O$. The effect of this chemical on catalase activity was similar to that of its homologue, ethylene oxide, in that it increased the activity of potted plants. The results of the treatment of potted plants were as follows: (.01 M) 14.7, 14.8, 18.2, and 25.4 per cent; (.1 M) 14.0, 18.7, 19.1, and 44.0 per cent; (1.0 M) 13.9, 28.3, 28.6, and 29.0 per cent. The appearance of the plants was the same as that of those treated with ethylene oxide.

The treatment of tissue preparations resulted in declines in catalase ac-

tivity as follows: (.01 M) — 1.8 and — 2.9 per cent; (.1 M) — 7.3 and — 7.8 per cent; (1.0 M) — 9.8 and — 10.9 per cent.

The toxicity of propylene oxide, according to Harvey (1931), is similar to that of ethylene oxide. This worker noted that when woody plants were killed with either of these chemicals there was a darkening of the inner bark. He thinks this may be the result of an increased activity of the oxidizing enzymes.

Sodium arsenite. Na_2HAsO_3 . Potted plants treated with this chemical showed changes in catalase activity as follows: (.01 M) 20.6, 31.9, 33.7, and 66.7 per cent; (.1 M) — 4.8, — 7.0, — 11.5, and — 24.6 per cent; (1.0 M) — 26.1, — 38.9, — 63.0, and — 69.0 per cent. It will be noted that treatment with the .01 M concentration was followed by increases in catalase activity.

The leaves of the plants given the .01 M treatment showed a slight loss of color. The .1 M concentration caused a slight wilting and mottling of the leaves. The leaves of the plants receiving the 1.0 M treatment were badly wilted and much mottled. Mottling of the leaves seemed to be characteristic of arsenic injury.

The results of the tissue preparation treatments were: (.01 M) — 1.2 and — 1.4 per cent; (.1 M) — 5.4 and — 6.3 per cent; (1.0 M) — 70.5 and — 74.8 per cent.

Stewart and Smith (1922) mention the stimulatory effect of minute quantities of arsenic on growth. They are of the opinion that this may result from the destruction of harmful soil organisms or be a direct physical-chemical effect. Euler and Blix (1919) found that low concentrations of certain protoplasmic poisons would considerably accelerate the catalase activity of yeast cells.

The toxic action of arsenic appears to be that of a protoplasmic poison. According to Warburg (1925), the toxicity of arsenical compounds is partly due to their affinity for iron. Catalase activity has been considered by some workers as associated with oxidation processes. Lyon (1917) thinks that arsenic may be an oxidation catalyst. Should this be the case, it may account for the acceleration in catalase activity shown by the plants given the .01 M treatment.

Sodium chlorate. NaClO_3 . This commonly used herbicide was found to be the least effective of the chemicals that reduced catalase activity. The following changes occurred in the catalase activity of potted plants: (.01 M) 5.5, 1.4, — 3.8, and — 5.7 per cent; (.1 M) — 6.3, — 8.1, — 9.0, and — 11.8 per cent; (1.0 M) — 6.8, — 10.5, — 14.0, and — 23.3 per cent. The plants given the 1.0 M concentration were the only ones to show any evidence of injury 24 hours after treatment. These plants were slightly wilted.

Tissue preparations treated with sodium chlorate showed the following decreases in catalase activity: (.01 M) — 5.7 and — 10.4 per cent; (.1 M) — 11.4 and — 15.3 per cent; (1.0 M) — 24.0 and — 26.4 per cent. Neller

(1931) found that the addition of this chemical to bindweed root preparations had little immediate effect on their activity.

The toxicity of sodium chlorate has been ascribed to its high oxygen content. Harvey (1931) states that the salt has an oxidizing effect on the respiratory chromogens. Offord and d'Urbal (1931) found that sodium chlorate caused the outward diffusion of substances from the alga *Nitella*. This would indicate a change in cell permeability.

TABLE I. *Averaged recapitulation of the data presented*

Chemical	Material treated	Concentration of treatment and percentage of change in catalase activity		
		.01 M	.1 M	1.0 M
Ammonium sulfocyanate	4 Plants	0.25	—7.5	—84.5
	2 Tissue prep.	—10.9	—64.1	—79.5
Thiourea	4 Plants	—25.4	—65.8	—78.7
	2 Tissue prep.	—11.1	—20.0	—37.9
Ethylene oxide	4 Plants	24.8	30.3	27.3
	2 Tissue prep.	0.0	—2.7	—23.4
Diethylene oxide	4 Plants	—7.2	—12.1	—21.5
	2 Tissue prep.	—4.7	—11.7	—33.3
Propylene oxide	4 Plants	18.3	23.9	24.9
	2 Tissue prep.	—2.3	—7.5	—10.3
Sodium arsenite	4 Plants	38.2	—12.0	—49.2
	2 Tissue prep.	—1.3	—5.8	—72.6
Sodium chlorate	4 Plants	—0.6	—8.8	—13.6
	2 Tissue prep.	—8.0	—13.3	—25.2

SUMMARY

An accurate and rapid method of making catalase determinations is described.

The toxicity of a chemical cannot be determined accurately from its effect on the catalase activity of tissue preparations.

The toxicity of a chemical cannot be predicted from its similarity to compounds of known toxicity.

The measurement of changes in catalase activity is useful in studying the herbicidal properties of chemicals, provided the toxic action peculiar to the chemical being investigated is taken into consideration.

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CHROMOSOMES AND COMPATIBILITY IN THE ALOINAE

ALFRED MARSHAK

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In the course of some cytological investigations data were obtained on chromosome number and form in the Aloinae which may be of interest to the systematist.

Taylor (1925) has made careful comparisons of the chromosome morphology in some species of *Aloë*, *Gasteria*, and *Haworthia*, and has found as the haploid complement in all, four long and three short chromosomes. In *Gasteria* all but one of the large chromosomes have subterminal constrictions. The odd one has its constriction somewhat further removed from the end and bears a "satellite" at the distal end. *Haworthia* is essentially like *Gasteria*, but here the odd chromosome does not bear a "satellite," and two of the others give indications of a secondary constriction in the short "arm" of the chromosomes. In *Aloë arborescens* Mill. and *A. saponaria* Haw. three of the large chromosomes resemble the odd one of *Haworthia*; the fourth, which bears a satellite, has the fiber constriction more nearly terminal—i.e., like the three of *Gasteria*.

Ferguson (1926) has investigated a number of species in four different genera of the Aloinae. Four long and three short chromosomes constitute the usual haploid complement in all the genera she observed, although there is a tetraploid species in each. She did not find the particular distinguishing features described by Taylor. A similar complement of five long and twenty-five short pairs of chromosomes has been described in the Central American genera *Yucca* and *Agave* (O'Mara, 1931; McKelvey and Sax, 1932).

In the present investigation descriptions are based only on figures from the first pollen grain division. It is possible that chromosomes are more "contracted" here than in the somatic divisions, but the essential features of their morphology seem clear, especially since they tend to lie flat on the equatorial plate, which is usually not the case in somatic divisions. Smear preparations were used exclusively. These were fixed and stained in aceto-carmine, aceto-haematoxylin, and chrom-acetic-osmic followed by crystal violet.

In *Aloë grandidentata* the writer has observed four long and three short chromosomes. Sax found a similar condition in *A. striata*. The *A. vera* examined showed three long, one intermediate, and three short chromosomes.

The following species of *Gasteria* were found to have the usual complement: *G. brevifolia*, *G. carinata*, *G. disticha*, *G. elongata*, *G. lingua*, *G. mollis*, *G. nigricans*, *G. planifolia*, *G. pulchra*, *G. subcarinata*, *G. sulcata*, *G. verrucosa*.

The *Haworthia* species showing the same type of complement were: *H. attenuata*, *H. planifolia*, and *H. tortuosa*.

Occasional pollen grains in *H. planifolia* and *H. brevifolia* showed an additional chromosome. A few pollen grains of *G. sulcata* lacked one of the short chromosomes. In this species there was an unusual amount of abnormal pollen, which may have been due to the high temperature at which the plants were grown. *Apicra congesta* showed similar variability in chromosome number, but the usual count was six long and three short. In *Aloë vera* one of the long chromosomes is replaced by one of intermediate length. No evidence was obtained of "satellites," of consistent differences in large and small "lobes," or of "double constrictions" comparable to those found by Taylor. It should be remembered, however, that these features were observed most clearly, if not altogether exclusively, in somatic mitoses.

One hesitates to draw inferences about wild species from representatives grown so long under cultivation. According to Baker (1880-1881), twenty of the Cape species had been introduced into European gardens as early as the seventeenth century. However, it is of interest to note that *Aloë vera*, whose complement differs from that characteristic of the group, not in a deficiency or excess of any of the usual types of chromosomes, but in a decided difference in the length of one of the long chromosomes, has also an unusual geographic distribution. Unlike the other aloes, which are almost exclusively African (with a few in southern Arabia), it is found in the Mediterranean region extending west to the Canaries and east to China and Formosa. Berger (1908) considers that such a wide distribution has been made possible through the intervention of man. However, even if *A. vera* is not considered indigenous to these regions, it is striking to note that it alone should have been able to survive and that there is correlated with this different survival value a difference in its chromosomal constitution. It seems worth while to distinguish between such differences and those in which there is apparent duplication of the complement. *Apicra congesta* suggests a third class in which individual chromosomes may have been duplicated. The condition in *Apicra*, however, is doubtful because of the marked variability of the pollen observed. Disregarding this case, it seems clear that rather small differences in chromosome size (or shape) are associated with marked phenotypic differences such as those between *A. vera* and *A. grandidentata* and, accepting Taylor's findings, between *Aloë*, *Gasteria*, and *Haworthia*.

Berger noticed in the aloes that although the anthers reached the level of the stigma and that self-pollination therefore seemed inevitable, nevertheless, selfed flowers never produced seed. "*Aloë aethiopica*, *pluridens*, *caesia*, etc., setzen selbst nach künstlicher Bestäubung mit Blüten desselben Stockes keine Kapseln an. Sonst sind jedoch die *Aloë* und ihre Hybriden sehr fruchtbar." Without previous knowledge of this the writer noticed a similar condition in *Gasteria* and made self- and cross-pollinations in an effort to detect the presence of factors for self-sterility. The results with eight species are as follows:

Self-fertile—*G. lingua*, *disticha*, *sulcata*

Self-sterile—*G. brevifolia*, *nigricans*, *planifolia*, *pulchra*, *verrucosa*

All species of both classes are interfertile. The pods set by the self-fertile plants are small and contain about a dozen seeds, while pods from crossed blossoms are large, with forty-five to fifty seeds. The plants of the first group may possibly be better classed as pseudo-fertile (East and Yarnell, 1929). Numerous self-pollinations with four species of *Haworthia* proved fruitless. Likewise nineteen reciprocal pollinations between four species of

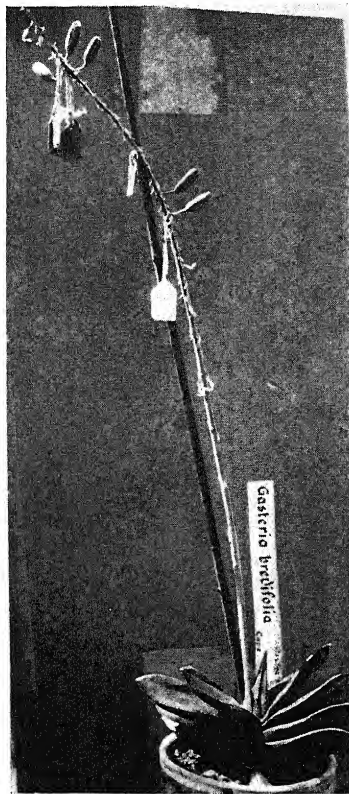


Fig. 1. *G. brevifolia*, showing pods set only where cross pollination has occurred.

Haworthia and two of *Gasteria* produced no seed. Although the tests are as yet by no means adequate, they indicate a series of sterility factors in both genera. Failure of these intergeneric crosses can hardly be due to gross chromosome differences. It might be attributed to the accumulation of mutations (Sax, 1931), to rather small differences in chromosome morphology such as Taylor has described, or to translocations (Brink, 1932). No rings or chains of chromosomes were observed in the species examined. Since

intergeneric hybrids have not been obtained, a suitable test of the latter hypothesis cannot be made. Some agency segregating mutants is prerequisite for the accumulation of genetic differences. In the absence of geographic isolation as in the present case, some other mechanism preventing intercrossing such as different time of flowering or different physiological condition of the pistil must be assumed. With the presence of self-sterility factors incompatibility due to a single pair of genes cannot be overlooked as a possible agent in the segregation of types. However, further discussion of the situation seems useless until more information concerning sterility factors in each of the genera is obtained.

SUMMARY

Two species of *Aloë*, twelve of *Gasteria*, and three of *Haworthia* were found to have chromosome complements similar as to number and morphology. There are four long and three short chromosomes. *Aloë vera* is exceptional in having three long, one intermediate, and three short chromosomes. This species also has a much wider geographical distribution than other members of the group.

The presence of self-sterility factors in species of *Gasteria* and *Haworthia* is pointed out.

The writer wishes to thank Dr. William Crocker and Miss Lela V. Barton of the Boyce Thompson Institute for their kindness in making germination tests of seeds from the various crosses.

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EXPLANATION OF PLATE I

Figures 1-8 ($\times 2330$) and 9-12 ($\times 1750$) were drawn with different sets of lenses. Direct measurement of chromosomes of the different genera with the same optical system and drawings with the same camera lucida showed that the long chromosomes in all were of the same size.

1. *Aloë grandidentata*. 2. *Aloë vera*. 3. *Aloë vera*. 4. *Gasteria lingua*. 5. *Gasteria planifolia*. 6. *Gasteria nigricans*. 7. *Gasteria sulcata*. 8. *Gasteria brevifolia* (unusual pollen grain with 4 small chromosomes). 9. *Apicra congesta*. 10. *Haworthia attenuata*. 11. *Haworthia planifolia*. 12. *Haworthia planifolia* (unusual pollen grain with 5 long chromosomes).



MARSHAK: ALOINAE

ANNUAL RING FORMATION IN *PINUS PALUSTRIS* SEEDLINGS

L. J. PESSIN

(Received for publication May 15, 1933)

Longleaf pine (*Pinus palustris* Miller) in its seedling stage differs markedly from all the other pines indigenous to the Southern States. When the longleaf pine seed falls on the ground during October and November, it does not lie dormant until the following spring as do the seeds of the other pines, but in two or three weeks, if weather conditions are favorable, the seed germinates.

Three definite stages in the development of the seedling can be observed. The first stage is characterized by the presence of the cotyledonary or seed leaves, which function for only a few months. These are succeeded by the primary leaves, which arise on the main axis, and after several months may disappear or be transformed into permanent scales. This second stage usually terminates at the beginning of the second spring, when secondary or foliage leaves appear in the axils of the primary leaves. The third stage then follows, in which the foliage leaves are fully developed and are morphologically distinct from the primary leaves. At this time the main axis of the stem is hidden by the central tuft of the foliage leaves. The growth of the stem is very slow and hardly noticeable. In this condition the seedling may remain for several years, showing very little increment in height or diameter. In the other southern species of pine the seedlings begin height growth soon after germination; such a stand of seedlings, now 12 years old, is under observation in southern Louisiana. The roots of the longleaf seedlings, however, grow rapidly, resulting at the end of 3 or 4 years in a well developed root system. Seedlings in this stage—that is, when the needles arise from a central cushion-like region and not directly from a terminal, well developed bud—are said to be in the “grass stage,” for at this time they are practically indistinguishable from the surrounding grasses. Only an experienced eye can detect a longleaf pine seedling in a stand of green grass. During the “grass stage” there appears to be no marked active and dormant season in the growth of longleaf seedlings. Even during the winter months if weather conditions are favorable, new needles are often seen arising from the central cushion-like region.

After several years, depending on individual seedlings and on site conditions, the main axis, which up to that time has been hidden by the central tuft of needles, begins to rise and ultimately forms a “pointed” bud. When this becomes the characteristic fuzzy, silvery longleaf pine bud, the third stage is

ended. From then on the stem of the seedling grows in height and in diameter. At first the bud elongates, forming what is commonly known as the "candle." Later, clusters of needles arise from well developed fascicles all along the new stem. This marks the beginning of a definite growing season, with a slowing-down of growth in the winter and a marked increase in the spring and summer.

An anatomical study was made of the stems and roots of a number of longleaf pine seedlings. Two specimens, representative of different types of early development, are here described. The seedlings, growing within a foot of each other, were of the same age and originated from the seed crop of 1920, making them 12 years old at the time of the study. One seedling measured 7.5 cm. high, from root collar to tip of stem, and had no definite bud; the foliage leaves arising from the central cushion-like region. The other seedling was 36.6 cm. high, with a well developed typical longleaf pine "pointed" bud. Sections 30 microns thick were cut from the stem and root at different heights, as illustrated in plates 1 and 2. These sections were photographed, using a magnification of 45 diameters. Plate 1 includes photographs of sections of the smaller seedling, and in plate 2 are shown the photographs of the sections of the taller seedling taken at different heights.

An examination of the photographs shows that the stem of the taller seedling, with a typical bud, has annual rings to within 5 cm. from the tip, while the smaller seedling, with no definite bud, although of the same age, has no annual rings. It is evident that actual cambial activity begins only after terminal buds are developed. This confirms a statement by Priestley¹ that "cambial activity on the surface of the old wood begins beneath the bud and spreads basipetally down the tree." He also found that "cambial activity spreads downward with great rapidity from the buds."

In the longleaf pine there is no definite active growing and dormant season while the seedling is in the "grass stage" and no annual rings are developed during this stage. As soon as a typical terminal bud appears, definite growing and dormant seasons result and the seasonal cambial activity results in annual rings.

The number of rings observed on a cross section of a longleaf pine at ground level does not indicate the total age of a longleaf pine. It merely shows the number of years since a terminal bud was first formed. How long before that the seedling remained in the "grass stage" is, of course, impossible to determine accurately, since individual seedlings behave differently in this respect.

SUMMARY

1. The stem of the seedling of *Pinus palustris* grows very slowly during the first few years following germination, but the root system is well developed by the time the seedling is three or four years old.
2. The period of slow height growth depends not only on conditions of

¹ Priestley, J. H. 1932. The growing tree. *Forestry* 6: 107-112.

habitat, but also on the individual seedling. Some start height growth in four years, while others may remain in the "grass stage" for 12 years or more following germination.

3. While the seedling is in the "grass stage," no distinct annual rings are evident either in the root or in the stem. Spring and summer wood are distinguished only after true terminal buds have been formed. Then the growing season becomes definite, with rapid growth in the spring and a slowing-down in the fall. Prior to this, growth may occur at any time, even during the winter months, resulting in no definite active and dormant seasons.

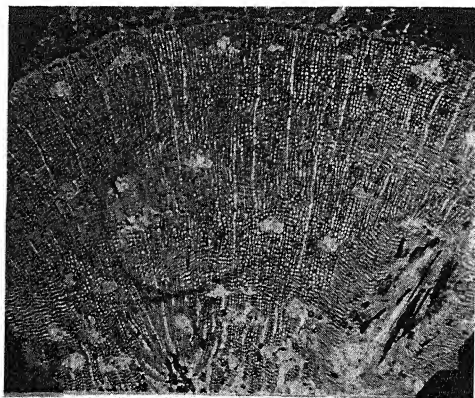
4. A count of the annual rings in the stem of a longleaf pine tree at a given height does not indicate the age of the tree at that point on the stem from the time of the germination of the seed. It shows merely the number of years that have elapsed since the stem began to grow in height. How long the seedling remained in the "grass stage" before height growth started cannot be accurately determined.

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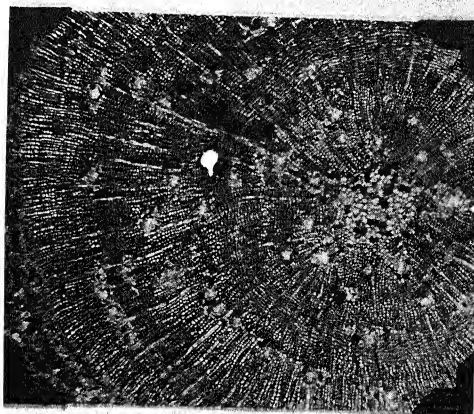
EXPLANATION OF PLATE I

Seedling of *Pinus palustris*, 12 years old, 7.5 cm. high, with no definite terminal bud.

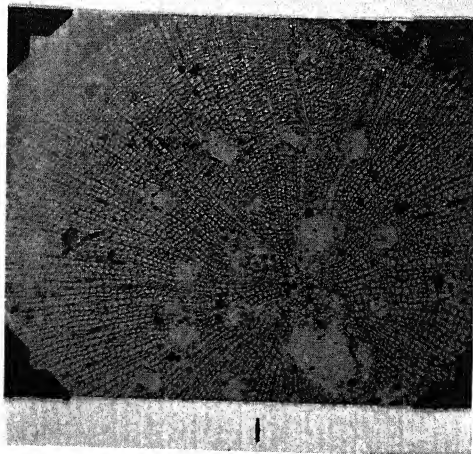
Fig. 1. Cross section of root 5 cm. below root collar, showing lack of annual rings. $\times 45$, 30μ thick. Fig. 2. Cross section of stem at root collar, showing lack of definite annual rings. $\times 45$, 30μ thick. Fig. 3. Cross section of stem 2.5 cm. above root collar, showing lack of definite annual rings. $\times 45$, 30μ thick.



3

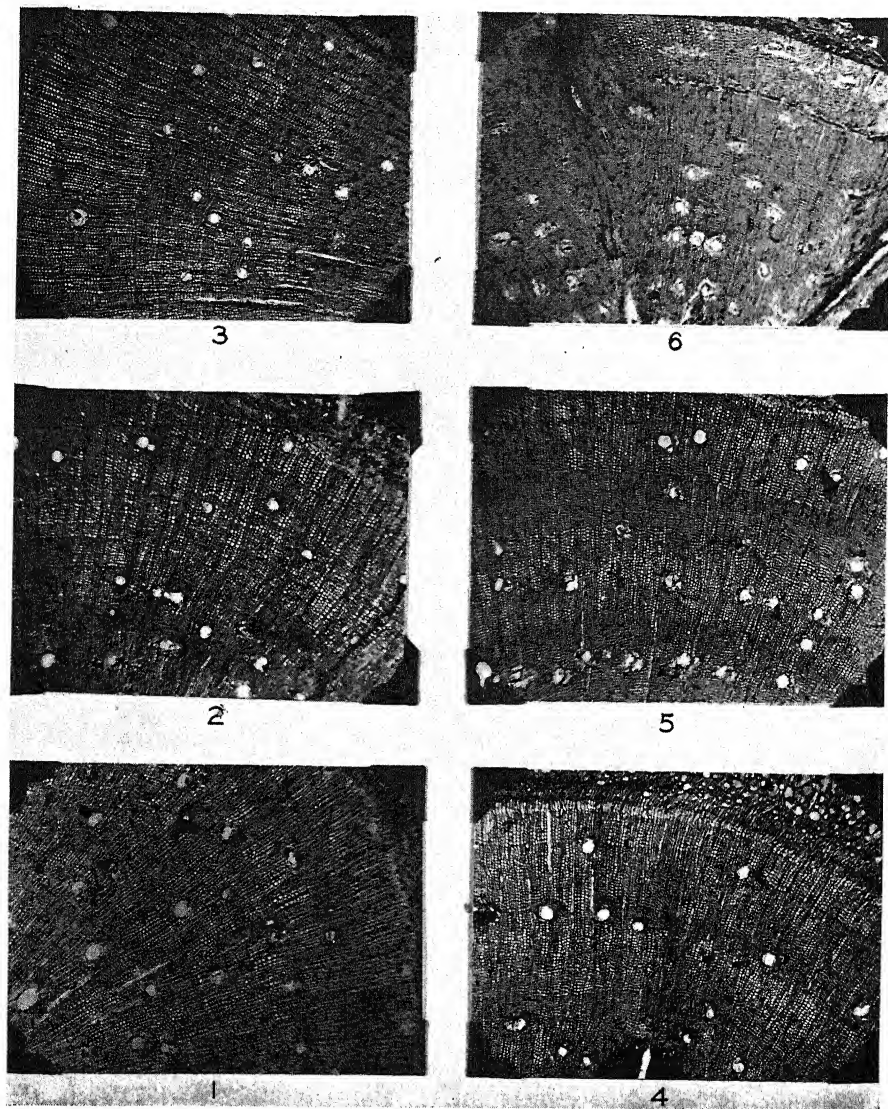


2



1

PESSIN: PINUS PALUSTRIS

PESSIN: *PINUS PALUSTRIS*

EXPLANATION OF PLATE 2

Seedling of *Pinus palustris*, 12 years old, 36.6 cm. high, with well developed terminal bud.

Fig. 1. Cross section of root 1.3 cm. below root collar, showing definite annual rings. $\times 45$, 30μ thick. Fig. 2. Cross section of stem at root collar, showing definite annual ring. $\times 45$, 30μ thick. Fig. 3. Cross section of stem 2.5 cm. above root collar, showing definite annual rings. $\times 45$, 30μ thick. Fig. 4. Cross section of stem 7.7 cm. above root collar, showing definite annual rings. $\times 45$, 30μ thick. Fig. 5. Cross section of stem 15.3 cm. above root collar, showing definite annual rings. $\times 45$, 30μ thick. Fig. 6. Cross section of stem 30.6 cm. above root collar (current growth), showing lack of definite annual rings. $\times 45$, 30μ thick.

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FURTHER STUDIES ON THE RELATION BETWEEN THERMAL EMISSIVITY AND PLANT TEMPERATURES¹

ALFRED N. WATSON

(Received for publication April 29, 1933)

There has been much discussion of the importance of transpiration as a method of dissipation of radiant energy absorbed by leaf tissue. Brown and Escombe (1905), observing that in their experiments transpiration sometimes accounted for as much as 80 per cent of the total heat given off during a temperature change in the leaf tissue, concluded that transpiration was of considerable importance in preventing leaf tissue from overheating. Shull (1919) considered transpiration to be "vitally necessary." Clum (1926), after extensive work on transpiration and leaf temperatures, found no correlation between them. He also concluded that since great temperature changes occurred in the leaf within a few seconds, transpiration could not be the major factor operating to produce such changes.

In a previous paper (1933) the writer considered the various factors which cause measurable thermal exchanges in leaf tissue and concluded that transpiration and thermal emission from the leaf surfaces were the only significant ones. Thermal emissivity is there defined as the number of calories of heat given off per minute from a square cm. of leaf surface for each degree temperature difference between the leaf and air. Thermal emission is therefore a physical property of the leaf, independent of transpiration or other thermal factors.

In determining thermal emissivity, transpiration must be entirely eliminated. This may be done indirectly if the mass, specific heat, area, and rates of temperature loss for various temperature differences are known.

Brown and Escombe obtained a value for thermal emissivity of *Liriodendron tulipifera* leaves by a differential transpiration method involving temperature differences between the leaf and atmosphere of only 2 C. degrees. In experiments with the same species the author found varying and conflicting results up to temperature differences of 3 C. degrees but consistent results with temperature differences between 3 C. degrees and 30 C. degrees. At ordinary room temperatures (about 20 C. degrees) the upper temperature difference involves a leaf temperature of 50 C. degrees, which closely approaches the thermal death point of this species. It was therefore concluded that thermal

¹ Papers from the Department of Botany, Ohio State University, No. 329.

[The JOURNAL for November (21: 513-604) was issued November 6, 1934.]

emissivity cannot be accurately measured unless the entire temperature range has been used. In these experiments it was found that a normally transpiring *Liriodendron tulipifera* leaf decreased in temperature at the rate of 2.1 C. degrees per minute for each degree that the leaf was above air temperature. As previously stated, the temperature differences below 3 C. degrees were not considered because such a high percentage of the dissipated energy was used in transpiration that thermal emission played a negligible rôle. The latter situation is probably the usual one during the life of the plant.

Meyer (1932a) has made investigations upon the daily periodicity of transpiration in the tulip poplar, *Liriodendron tulipifera*. His paper also contains data upon the fresh and dry weight per unit area of the leaves. For the purpose of this paper, only the average results obtained from seven trees are taken from Meyer's data.

Leaf area per tree	2.56 dm. ²
Fresh weight of leaves per tree	3.20 gm.
Fresh weight per dm. ² of leaf area	1.25 gm.
Dry weight of leaves per tree69 gm.

From these data it can be calculated that the leaves were composed of 22 per cent dry matter and 78 per cent water. The specific heat of the leaves is therefore:

$$(.78 \cdot 1) + (.22 \cdot .30) * = .85.$$

A temperature difference must exist before thermal emission can account for any loss of heat. The greater this difference, the greater is the proportion of heat dissipated by this method and the less by transpiration. We now have sufficient data to calculate the relative amounts of heat lost by thermal emission and transpiration for different transpiration rates and temperature differences for leaves of *Liriodendron tulipifera*.

The percentages in table 1 were calculated in the following manner. The amount of the total absorbed heat dissipated by thermal emission for a temperature difference between the leaf and air of 5 C. degrees and transpiring at the rate of .71 gm. per dm.² per hour is found in the table to be 38 per cent.

The calculation of this value was as follows: Let M be the mass of a dm.² of leaf surface. Let S be the specific heat of the leaf. Let θ be the temperature difference between the leaf and air. Let K be the rate of temperature loss per minute per degree temperature difference (Watson, 1933). Let W be grams of water lost by transpiration. Let L be the latent heat of vaporization of water at 25 C. degrees. The heat loss per minute per dm.² is

$$MS\theta K \text{ calories}$$

and per hour

* Meyer (1932b) and Chrysler (1931) place the specific heat of dry material at .30. Any error here is greatly reduced in multiplication by the factor .22 (percentage of dry matter of leaf).

TABLE 1. *Hourly variation in percentage of absorbed heat dissipated by thermal emission*

Temperature diff.—C. degrees	Transpiration rate—gm./dm. ² /hr.											
	.05	.05	.06	.29	.83	.93	.85	.71	.71	.42	.09	.04
4	95	95	94	71	10	1	10	25	25	55	90	96
5	96	96	95	76	28	18	25	38	38	63	92	97
6	96	96	96	81	40	33	39	49	49	70	94	97
7	97	97	96	83	49	43	48	56	56	74	94	98
8	97	97	97	85	55	50	54	62	62	77	95	98
9	98	98	97	87	60	55	59	66	66	80	96	98
10	98	98	97	88	64	60	63	70	70	82	96	98
11	98	98	98	89	68	63	67	72	72	83	96	98
12	98	98	98	90	70	66	70	74	74	85	97	99
	12-2	2-4	4-6	6-8	8-10	10-12	12-2	2-4	4-6	6-8	8-10	10-12
	Noon											

MSOK(60) calories.

Substituting

$$(1.25)(.85)(5)(2.1)(60) = 670 \text{ calories per hour.}$$

Heat lost by transpiration is

WL calories per hour.

Substituting

$$(.71)(580) = 412 \text{ calories lost per hour.}$$

Therefore the percentage of heat dissipated by thermal emission is

$$\frac{670 - 412}{670} = 38 \text{ per cent.}$$

Calculations for table 2 were based on the following equation:

$$\frac{\text{Total heat lost} - \text{Heat lost by thermal emission}}{\text{Total heat lost}} = \text{Percentage of heat lost by transpiration.}$$

Table 2 clearly demonstrates the point that transpiration accounts for more than 50 per cent of the heat loss only when both of the following conditions obtain: (1) the transpiration rate exceeds .71 gm./dm.²/hr.; (2) the difference in temperature between the leaf and air is less than 7 C. degrees.

TABLE 2. *Hourly variation in percentage of absorbed heat dissipated by transpiration*

Temperature diff.—C. degrees	Transpiration rate—gm./dm. ² /hr.											
	.05	.05	.06	.29	.83	.93	.85	.71	.71	.42	.09	.04
4	5	5	6	29	90	99	90	75	75	45	10	4
5	4	4	5	24	72	82	75	62	62	37	8	3
6	4	4	4	19	60	67	61	51	51	30	6	3
7	3	3	4	17	51	57	52	44	44	26	6	2
8	3	3	3	15	45	50	46	38	38	23	5	2
9	2	2	3	13	40	45	41	34	34	20	4	2
10	2	2	3	12	36	40	37	30	30	18	4	2
11	2	2	2	11	32	37	33	28	28	17	4	2
12	2	2	2	10	30	34	30	36	36	15	3	1
	12-2	2-4	4-6	6-8	8-10	10-12	12-2	2-4	4-6	6-8	8-10	10-12
	Noon											

An analysis of tables 1 and 2 shows the relative importance of thermal emission in the dissipation of heat. In the case of *Liriodendron tulipifera* leaves transpiring at a maximum rate, the leaf temperature, as shown by table 1, must rise 8 C. degrees above air temperature before 50 per cent of the heat is dissipated by thermal emission. However, were the transpiration rate greatly reduced, as in the case of incipient wilting, so that the minimum observed rate held, then at this same temperature difference—viz., 8 C. degrees—98 per cent of the heat would be lost by means of thermal emission.

SUMMARY

This paper is the result of application of certain previously derived formulae concerning the thermal emissivity of *Liriodendron tulipifera* to transpiration data of Meyer (1932a). The relative amounts of heat dissipated by thermal emission and transpiration were calculated for varying rates of transpiration and temperature differences between the leaf and air.

The evidence obtained in this investigation supports the conclusion that neither transpiration nor thermal emission can be called the more important factor. In any such discussion due consideration must be taken of the transpiration rates, the water content of the leaves, and the rate of energy absorption. All three of these factors must be known and the conclusion must be limited to the species in question under the observed conditions. No conclusions regarding the relative importance of transpiration can be drawn if based

only upon the total amount of heat dissipated by that means. Such conclusions have often led past investigators to overemphasize the importance of transpiration. However, as table 1 shows, for any given transpiration rate, the rôle of thermal emission becomes increasingly important with increase in temperature difference between the leaf and air. Thus, even under high rates of transpiration, it is the deciding factor in preventing the thermal death point of the leaf from being reached under conditions of excessive incident and absorbed radiant energy.

This is the second paper of a series dealing with the relation between thermal emissivity and plant temperatures. The writer wishes to express his appreciation to Dr. E. N. Transeau of the Ohio State University for guidance and helpful suggestions, to Dr. B. S. Meyer for valuable criticism of the completed manuscript, and to Dr. F. C. Blake of the Department of Physics and Dr. R. Bradfield of the Department of Soils, through whose courtesy the thermocouple equipment was obtained.

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THE DEVELOPMENT OF SPHAGNUM BOGS IN THE SAN JUAN ISLANDS

GEORGE B. RIGG AND CARL T. RICHARDSON

(Received for publication May 9, 1933)

This paper gives the profiles of eight sphagnum bogs and discusses their development in postglacial times, emphasizing the general course of bog development in the San Juan Islands by pointing out the features that all the bogs have in common.

The San Juan Islands are a part of the State of Washington. The most northerly ones are a little south of 49° N. lat. They lie between the mainland of Washington and the south end of Vancouver Island. The distance from them to the Pacific Ocean through the Strait of Juan de Fuca is about 75 miles. The Islands are rocky and hilly and have several low mountains, from 1000 to 2500 feet high. Though their surface is largely rocky, there are considerable areas of glacial till and a number of swamp, muck, and peat areas. Their most characteristic vegetation is coniferous forest whose trees are smaller than those characteristic of the Puget Sound region in general. Lakes are common. Many of these have rocky shores, though some are partly bordered by swamps.

The weather in the Islands is mild. The summers are usually rather dry and cool, and the winters are rainy but not excessively cold. The average rainfall at Olga on Orcas Island over a period of years is 29.9 inches, 79 per cent of which falls in the 7 months from September to March. The average for July is 0.7 and for August 0.9. The variation from year to year is indicated by the high (36.02) in 1932 and the low (15.04) in 1929. The only other place in the Islands for which data are available is Friday Harbor, and the records there cover only 1931 and 1932. The average for these two years is 3.75 less than at Olga. The contours are such as to cut off the moisture-laden winds from the Ocean, and the Islands together with some portions of the mainland of Washington to the east and the southwest and the southeastern portion of Vancouver Island have lower rainfall than that of other portions of Western Washington and British Columbia. Seattle has an average rainfall of 33.40 for a period of 52 years, and points farther south have a still larger rainfall. Precipitation in excess of 100 inches occurs at several points on the west coast of Washington and Vancouver Island. It is thus apparent that the San Juan bogs have had, at least during the very recent years of their development, somewhat drier climatic conditions than most of the other bogs of Western Washington and British Columbia.

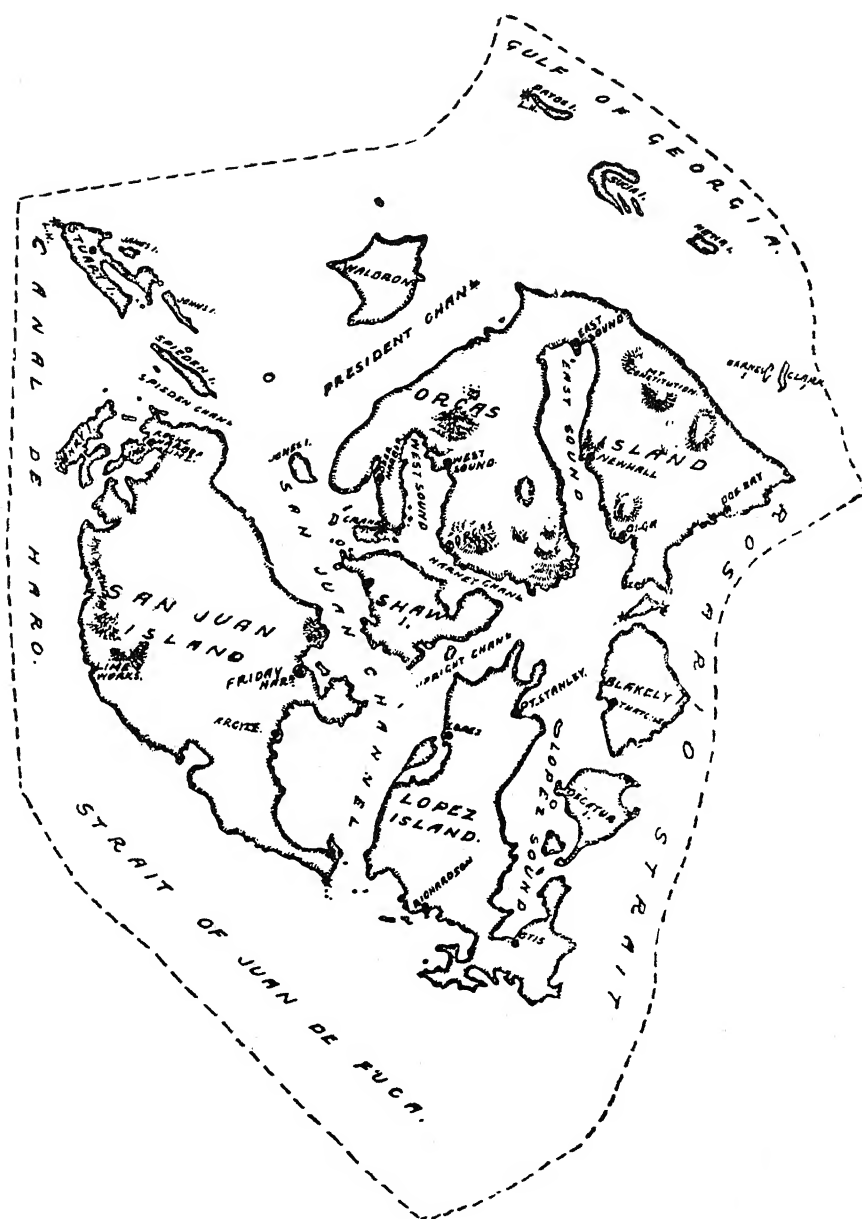


Fig. 1. Map of San Juan County, Washington.

The eight bogs discussed are all the sphagnum bogs that the authors have found in the Islands, though there are other peat areas that contain no sphagnum. The senior author has studied some of these bogs and the development of floating mats on the lakes of the Islands at various times during a period of twenty years. The profiles here presented were all made by the junior author during the summer of 1932. The bogs are evidently all postglacial. Their present flora and general character are similar to those of other sphagnum bogs of the Puget Sound region which have been described by the senior author.¹ Where the surface is composed of living or dead sphagnum, the characteristic shrubs are *Ledum groenlandicum* and *Kalmia polifolia* accompanied by the woody prostrate vine *Oxycoccus oxycoccus*. Characteristic herbs are *Drosera rotundifolia* and *Eriophorum chamissonis*. In their more mature stages these bogs are invaded by stunted conifers. *Tsuga heterophylla* and *Pinus contorta* are the most common. *Thuja plicata* and *Picea sitchensis* occur occasionally and *Pseudotsuga taxifolia* rarely. A natural "marginal ditch" borders most of them, and in these ditches and the sedge area bordering the sphagnum, *Spirea Douglasii* is common.

No pollen analysis has been made in this study. Such observations as to the presence of pollen as were made were incidental to the determination of the characters of the various layers of peat. Two peat borers (the Swedish type and the American type) were used. Most of the work was done with the latter, which is a slightly modified Davis instrument. Only one profile was made in each bog, and all interpretations made must be considered in the light of the fact that more borings might show somewhat different relations of the strata.

INDIVIDUAL BOGS

San Juan bog is situated on San Juan Island about four miles northwest of Friday Harbor in sec. 5, twp. 35 north, range 1 west, Willamette Meridian. It has an area of about 15 acres, much of which has been burned so that only about five acres of bog vegetation now remain at the center. It is surrounded by rocky hills, some of which are covered with glacial soil. It has been drained to the depth of a few feet by a ditch, but rock was encountered so that the deeper portions could not be drained. A smaller basin characterized by sedges is situated on the side opposite this drainage and drains into the bog.

Blakely bog is the smallest of the eight, being only about 125 by 60 feet. It is situated on Blakely Island about $\frac{3}{4}$ mile from Thatcher post office, on sec. 3, twp. 35 north, range 1 west. Its elevation above sea level is about 300 feet. It is bounded by rocks on the east side and by flat marshy ground on the other sides. It has not been burned or drained, and the native vegetation has not been disturbed.

¹ Bot. Gaz. 55: 314-316, 1913. Pl. World 19: 310-325, 1916. Jour. Forestry 15: 726-739, 1917. Publ. Puget Sound Biol. Sta. 2: 195-210, 1919. Ecology 3: 207-213, 1922. Ecology 6: 260-278, 1925.

Cold Spring bog is located on Orcas Island, on the south slope of Mount Constitution in sec. 29, twp. 37 north, range 1 west. It is surrounded by rocky banks 10 to 20 feet high except for the single opening at the southeast side where there is partial drainage. It contains about 7 acres.

Constitution bog number 1 is in sec. 20, less than half a mile from Cold Spring bog, and like it, is surrounded by rock and is partially drained through a single opening. Its area is about 9 acres. It is practically in its natural state.

Constitution bog number 2 is near the summit of Mount Constitution, which has an approximate elevation of 2500 feet. It is somewhat circular in shape and has a diameter of about 350 feet. It has a pond in the center which is bordered by a characteristic floating mat of sphagnum, sedges, and bog shrubs. It has not been burned or drained, and the native vegetation has not been disturbed. Both of the Constitution bogs are in Moran State Park.

The two Orcas bogs are on Orcas Island near the town of Orcas. Number 1 is on sec. 14, twp. 36 north, range 2 west. It is a circular bog containing about 9 acres. It is surrounded by steep rocky slopes from which rise on one side a range of low mountains 1100 feet high. Number 2 is near-by on sec. 11, and its surroundings are similar. Its area is about 18 acres.

Killebrew Lake bog is on Orcas Island near the Orcas bogs, about $\frac{3}{4}$ mile north of Grindstone Bay. It is adjacent to Killebrew Lake, and the flat on which it lies has an area of about 30 acres, the bog being about 500 by 350 feet. It has rocky hills on three sides and a marsh and the lake on the other. It has been burned and pastured so that much of the bog vegetation has been destroyed.

CHARACTER AND ORIGIN OF THE VARIOUS STRATA

The story of the development of these bogs evidently begins with the retreat of the glaciers from the region. Each occupies a depression which was formerly a lake. How much sand and gravel may have been washed into the depressions now occupied by these bogs is unknown, but it is evident that blue clay settled from the water of the lake occupying each depression and formed a coating over much of whatever lay beneath. In some cases sand is mixed with the clay. In two borings (No. 1, Killebrew, and No. 2, Cold Spring) no sand or clay was found, and this correlates with the contours of the rocky bottom at these points.

The story of the organic development of these bogs thus begins with the deposit of lake mud mixed with organic material on the surface of the clay, sand, gravel, or rock. The lake mud, which forms the bottom stratum in all eight bogs and is continuous in all except on the rocky projection in Cold Spring bog, consists of mineral and organic matter which gradually settled from the lake. The organic matter consists of the remains of plants and animals which flourished in the lakes with possibly some similar material

brought in by drainage. Some of the organic material is, of course, so completely disintegrated that no structure is recognizable in it even under the microscope. The visible remains of organisms are all microscopic and consist of diatoms, algae, and sponge spicules. The diatoms are mostly Navicula-like, though Fragillaria-like and Chaetoceros-like remains are found in some cases. Diatoms are especially abundant in San Juan bog and sponge spicules in Cold Spring bog. The recognizable algal remains consisted of fragments of filaments. The lake mud in the various bogs varies from olive green to dark brown in color.

Samples of this stratum from San Juan bog lost an average of 82.4 per cent on drying in air and then to constant weight in a desiccator. The dried material lost 34 per cent of its weight on ignition. The resulting ash lost 20 per cent of its weight on treatment with hydrochloric acid. Determinations of total nitrogen on the dried material gave an average of 2.0 per cent.

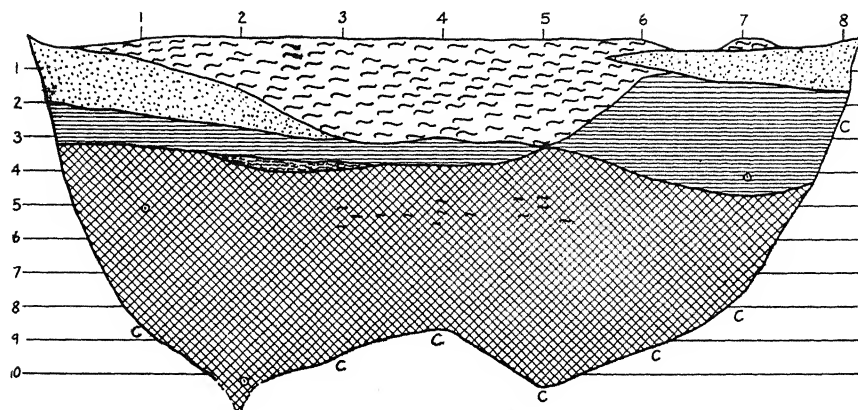
So far as the stratum of lake mud is concerned, we have no evidence that any organisms except small aquatic ones took part in its formation. No pollen grains were found in the samples examined. No extensive search for them was made, however, and their complete absence is not established. The source of the organic matter not recognizable under the microscope is, of course, undetermined, but it seems probable that the principal source of this organic material was phyto- and zoö-plankton with some sponges. This interpretation correlates well with observations on lakes occupying such depressions as these at the present time.

Sedge peat occurs in all eight bogs and lies directly on the lake mud in six of them. The plant remains visible to the unaided eye in this peat are portions of sedges, mosses, woody stems and roots, together with some seeds. Fragments of sedges are most common, being especially abundant in Cold Spring bog where the material becomes hard when dried and has good burning qualities. The mosses and wood consist of small fragments and the roots are very small. The seeds are dark colored with well preserved coats, but their interior is mostly disintegrated. The microscopic material consists of algae, pollen grains, and diatoms, the first two being abundant and the last rather scarce. The color of some of this peat is olive green, but it varies to lighter and darker colors. Extractions of the olive green material were made

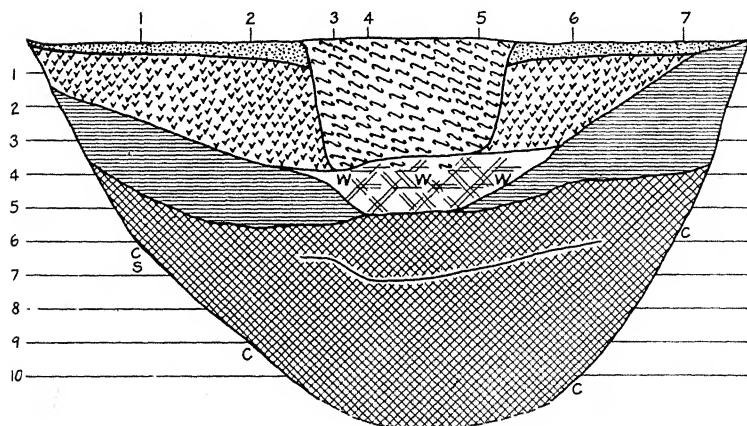
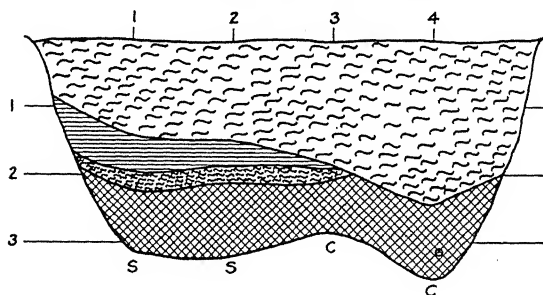
Fig. 2 (above). Profile of San Juan bog. The symbols for all peat strata that occur in any of the bogs are given in this figure whether they occur in this bog or not. The same applies to the letters (C—clay; S—sand; R—rock) indicating the material on which the organic matter rests, the numbers at the left indicating depth in meters, and the numbers at the top indicating the locations of borings. Horizontal scale 10 times vertical.

Fig. 3 (center). Profile of Blakely bog. For explanations see legend of fig. 2. Horizontal scale 1.5 times vertical.

Fig. 4 (below). Profile of Orcas bog number one. For explanations see legend of fig. 2. Horizontal scale 5 times vertical.

**LEGEND**

- | | |
|-------------------------|---------------|
| Sphagnum Peat 1-4 | Wood Peat |
| Sphagnum Peat 5-10 | Lake Mud |
| Sedge Peat | Muck |
| ◦ Volcanic Ash | Pondweed Peat |
| G - Gelatinous Material | |



with acetone and transferred to ether. The ether solutions showed a greenish color and when viewed through the spectroscope, they showed absorption bands indicating the presence of traces of chlorophyll and carotin.

This sedge peat is evidently the product of floating mats which grew on the surface of the lakes and eventually sank to the bottom. In the six bogs in which this material lies directly on the lake mud, it seems evident that these mats of sedges and other plants formed the first abundant community of macroscopic plants on the lakes. In Blakely bog the sedge mat development followed the growth of a layer of sphagnum, now represented by a thin layer of material in an advanced stage of disintegration, and in Killebrew Lake bog it came still later in the succession.

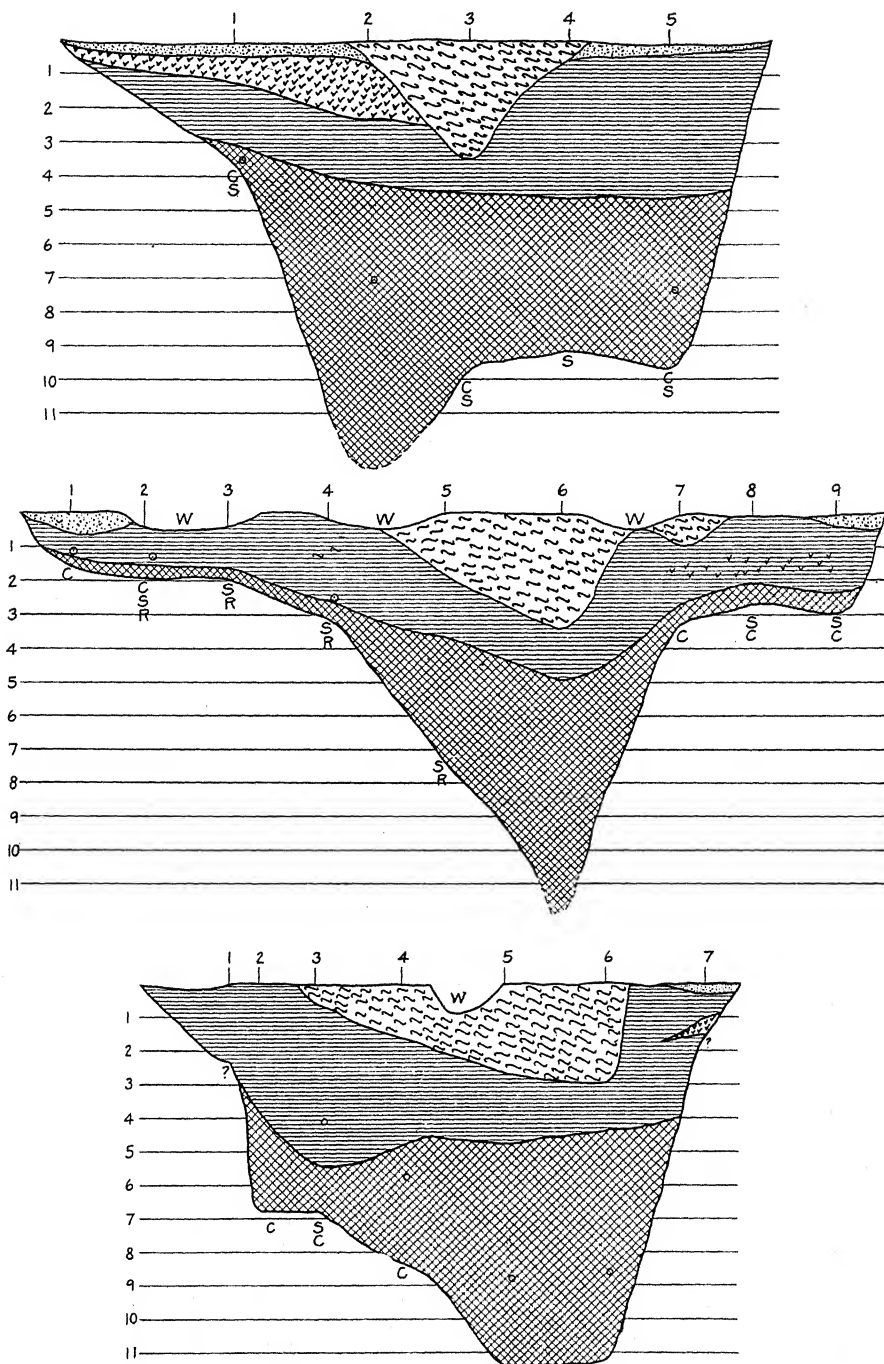
The stratum designated as pondweed peat forms a continuous layer over the lake mud in Killebrew Lake bog and does not occur in any of the others. The macroscopic material in this layer consists largely of bits of tissue from various aquatic plants, mainly pond weeds, though some well-preserved pieces of the culms and rhizomes of sedges are present. The microscopic material consists of diatoms of numerous species, spicules of fresh water sponges, and the pollen grains of several conifers among which are *Abies*, *Pinus*, and *Larix*. This stratum indicates that the first succession of macroscopic plants in this bog was different from that in the other bogs. The plant remains indicate that there must have been a dense growth of submerged plants, such perhaps as is common in lakes of the Puget Sound region at the present time. The presence of the remains of sedges seems to indicate that mat formation occurred to a limited extent at the time that this submerged vegetation flourished, and this may well be correlated with the presence of the very thick stratum of sphagnum just above it.

The presence of the stratum of gelatinous material within this layer of pondweed peat in Killebrew Lake bog presents an interesting problem. Although it appears gelatinous, it is also somewhat rubbery in character. It has a high water content and its color is yellowish or grayish. It appears to be in a colloidal state. A very thin layer of similar material was found at the bottom of the underground reservoir of water in the middle of Orcas bog number 1. There is a layer of similar material over 3 meters thick in a bog near Seattle (Cottage Lake bog number 2) lying between the lake mud and the sedge peat. The suggestion is very tentatively made that this material is the product of the action of micro-organisms on organic material after it was submerged in the lake, but a study of the physical and chemical properties of this material and any organisms that may be present in it must be made before we can speak with any confidence of its origin.

Fig. 5 (above). Profile of Orcas bog number two. For explanations see legend of fig. 2. Horizontal scale 10 times vertical.

Fig. 6 (center). Profile of Constitution bog number one. For explanations see legend of fig. 2. Horizontal scale 10 times vertical.

Fig. 7 (below). Profile of Constitution bog number two. For explanations see legend of fig. 2. Horizontal scale 5 times vertical.



Wood peat is present as a distinct layer in four of the bogs and is much mixed with the sedge peat in another one. In most cases it consists of particles of wood suspended in water and is thus of such a consistency that it is difficult to obtain samples with the peat borer. In a few cases, however, it was less watery and formed a fairly compact layer. Occasionally large pieces of wood, soft enough to be penetrated with the peat borer, but still showing distinct structure, were encountered. It is possible, of course, that considerable woody material may have been washed into the lakes from the surrounding forests, but the fact that most of the wood peat lies directly over the sedge, pondweed, or sphagnum peat probably indicates that a community of woody plants invaded the mats and sank with them. This view is strengthened by the fact that some mixture of wood with the sedge peat was found in nearly all of the bogs. That the woody plants were trees rather than shrubs is suggested by the large quantities of wood present and the large size of some of the pieces encountered in the borings. If this view is correct we may inquire the cause of the invasion of these mats by trees. We see three possible answers to this question. One is that the invasion occurred as soon as the mat was thick enough to furnish conditions suitable for the germination of the seeds and to support for a time the weight of the resulting plants. Another is that lowering of the lakes due to changes in drainage brought about conditions favorable to tree invasion. The third is that a change of climate favoring tree growth in the region was the determining factor. The first of these views seems sufficient to account for all the facts shown in the profiles. The second may have been a factor, though the fact that the drainage in most of these bogs is over rock must be considered. However, obstruction of the drainage may have caused the level of these lakes to be higher in their earlier stages than in later times. The theory of climatic change as a limiting factor in various stages of the development of bogs has many supporters, but the evidence for it seems inconclusive so far as our study of the bogs of the San Juan Islands is concerned. What may be indicated by pollen analysis remains to be seen.

Muck is present in the margins of most of the bogs. It probably represents organic and inorganic material washed in from the bordering slopes mixed with some organic material from the bog area itself. The vegetation on these muck borders consists of the characteristic "marginal ditch" plants.

The sphagnum layer which forms at least a portion of the surface in all eight bogs consists at the surface of one or more species of *Sphagnum* together with some other mosses. Living sphagnum is commonly present except where some portion of the bog has reached a very mature stage or where it has been modified by man's activities. The mosses, other than sphagnum, identified in these bogs are *Hylocomnium proliferum*, *H. triquetrum*, *Dicranum scoparium*, *Ceratodon purpureus*, and *Bryum pallescens*. The last mentioned is abundant with *Marchantia polymorpha* on the burned portion of San Juan bog close to the margin of the unburned portion, and the

others were collected from Blakely bog. Beneath the surface is sphagnum mixed with small stems and roots and vegetable debris. The degree of disintegration in this material is 1 to 4 on the von Post scale. On this scale, 1 designates peat in which there is no disintegration of tissues and which yields only water when pressed in the hand, none of the solid material coming out between the fingers. At the other extreme, 10 designates peat in which cellular structure is no longer recognizable and the entire mass comes out between the fingers when pressed in the hand. The numbers 2 to 9 designate gradual transitions from one extreme to the other. In the two bogs which have a second stratum of sphagnum peat under the sedge peat, the deeper layer of sphagnum consists of material ranging from 5 to 10 on this scale.

It seems probable that the sphagnum of both layers originated as floating mats. The formation of mats composed largely of sphagnum is common in the region at present. Herbaceous plants such as *Comarum palustre* and *Menyanthes trifoliata* and woody plants such as *Ledum groenlandicum* and *Kalmia polifolia* grow out into the water and thus form supports on which sphagnum forms a thick, tough mat. It seems probable that where a stratum of sphagnum peat is covered by a layer of sedge peat, the sedges grew on the sphagnum mat until it became so heavy that it sank. Where the sphagnum stratum is thick and extends to the surface, it is quite conceivable that much of the deposit may be due to the gradual dropping of loosened material from the bottom of the mat. Certainly this is taking place in bog lakes in the Puget Sound region at the present time. To what extent these deposits of sphagnum peat are due to the sinking of mats and to what extent they are due to the dropping of material from the lower surface is undetermined.

Sphagnum may also have formed dense growths in shallow pools such as commonly occur in bogs. The vigorous growth of sphagnum in such pools, with no mixture of other plants except small algae, is common in bogs at present. Still another method of sphagnum accumulation may have been the growth of the various species of *Sphagnum* around the bases of plants growing on a sedge mat. This phenomenon is, of course, also commonly seen in bogs.

The layer of volcanic ash was found in all bogs except Cold Spring bog. Its thickness is usually 1 to 3 cm., but in one boring in San Juan bog it is 10 cm. In Blakely bog it was found in only one boring, and it was not found in all borings in any bog.

The data shown in the profiles are consistent with the interpretation that each depression now occupied by a bog was then occupied by a lake and that the ash settled in the water and now rests on what was then the bottom of the lake. Four methods of accounting for the irregular distribution of this material occur to us. (1) Wind may have caused the material to drift on the surface of the lake and thus accumulate at one side. (2) Slow currents in the lake due to the water flowing from the outlet and flowing in as streamlets at various points may have moved the floating material. (3) Rains may

have caused the ash to slide into the lake from steep slopes. (4) The ash may have fallen on floating mats which did not sink until years later and the material may thus have been scattered so that it now forms a less definite layer than it would if it had settled from open water.

That volcanic ash does to a certain extent float on water was demonstrated by sprinkling it on water in beakers. The ash used was collected by the senior author at Kodiak, Alaska, in 1913, following the eruption of Mt Katmai in 1912. The coarsest of this material sank at once, as did also

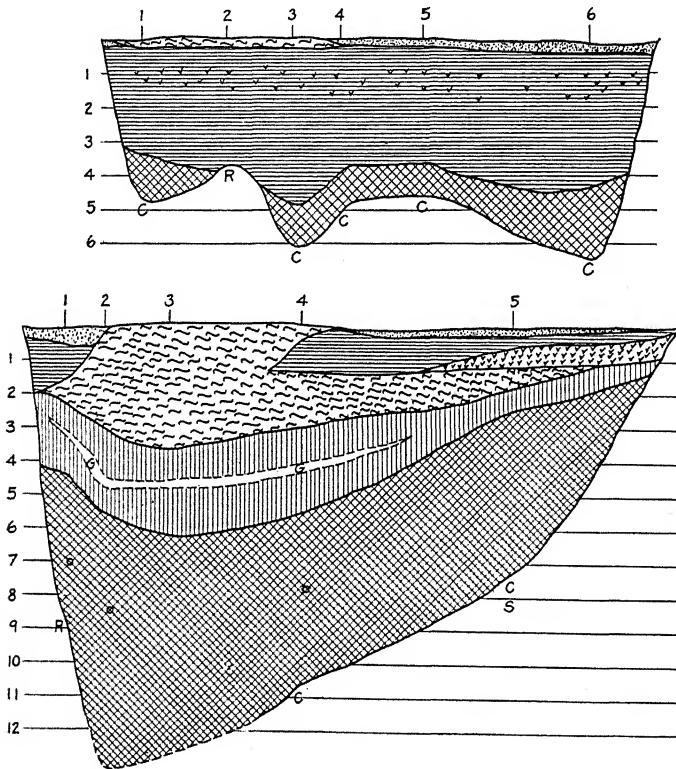


Fig. 8 (above). Profile of Cold Spring bog. For explanations see legend of fig. 2. Horizontal scale 10 times vertical.

Fig. 9 (below). Profile of Killebrew Lake bog. For explanations see legend of fig. 2. Horizontal scale 10 times vertical.

some of the finer, but a thin film remained on the surface for some time. Since very fine volcanic ash settles slowly from the atmosphere for at least several days following an eruption, it seems evident that either wind or water currents might account for much of the irregular distribution of this material. In most of the San Juan bogs, the layer was thicker toward the west or north-west side than at the opposite side, and this is consistent with the theory that

an east or southeast wind was a large factor in the distribution of the material on the lake bottom. The thick layer (10 cm.) in the west side of San Juan bog is, however, consistent with the theory that a stream flowing into the lake from the depression on the northeast side and another flowing out at the southwest was a large factor. To what extent ash may have slid into the lakes from steep slopes is unknown, but the extent to which sliding of wet volcanic ash into lakes and depressions occurred during rainy weather in Alaska following the Katmai eruption suggests such a possibility. That the ash did fall on floating mats in some cases is evident from its location in the profiles of the two Constitution bogs. It is evident, of course, that a single profile does not tell the complete story of the distribution of the ash in any bog. It is possible that the ash might be found in the sedge peat in other places by more borings.

If we assume, as seems reasonable, that the ash in all of the seven bogs in which it is present came from the same volcanic eruption, we have a date marked in these bogs which is unknown but is the same for all, and we thus have a means of comparing the stage of development of all of them at that time. On this basis we may say that so far as the data shown in the profiles are concerned, four of the areas now occupied by bogs were still in the lake mud stage at the time of the eruption. These are the two Orcas bogs, the neighboring Killebrew Lake bog, and Blakely bog. It seems that two bogs (San Juan and Constitution number 2) were mainly in the lake mud stage but had some sedge mats, and that one (Constitution number 1) may have been entirely covered by a sedge mat. Since no ash was found in Cold Spring bog, we have no evidence as to its stage of development. It is possible that ash might be found by making more borings. It is possible that it may have fallen on a sedge mat and thus have been so scattered before the sinking of the mat that it did not appear in the profile.

We have thus attempted to trace the story of the development of these bogs so far as the data at hand may indicate it, interpreting each stratum in terms of the plant communities that gave rise to it. The method used has been largely that of reasoning by analogy from facts about lake deposits and stages of bog development observed along the North Pacific Coast of America, together with comparisons of the literature of bog development in other portions of North America as well as Europe and Asia. Analogous reasoning may, of course, lead us into error, but it seems evident that all stages of the development of these bogs may be correlated with similar stages seen in bogs and lakes at the present time.

SUMMARY

Each of the San Juan bogs occupies a depression which was formerly a lake.

Three strata (lake mud, sedge peat, and sphagnum) occur in all.

Wood peat forms definite layers of varying extent in three bogs and is

abundantly mixed with the sedge peat in another. Fragments of wood are also commonly found in the sedge peat in all the bogs.

A stratum of pondweed peat lies directly on the lake mud in one bog.

Gelatinous material of unknown origin is present in two of the bogs.

Sphagnum forms all or part of the surface in all bogs and there is a separate, deeper layer of it in two bogs.

Volcanic ash occurs in all bogs but one. It is entirely in the lake mud in four, partially in the lake mud and partially in the sedge peat in two, and entirely in the lower portion of the sedge peat in one.

Muck is characteristic of the surface of the bogs at the margins and is more extensive in some.

It is probable that both the sedges and the sphagnum originated as floating mats.

Trees grew on some of these mats at some stage of the development of the bogs.

The location of the volcanic ash indicates that most of the bogs were in the lake mud stage at the time of the eruption but that three of them had formed some mats at the time.

The present vegetation of the bogs consists of characteristic herbs and shrubs, with forest succession in an early stage.

The various stages of development indicated by the strata shown in the profiles correlate with similar stages seen in lakes and bogs at the present time.

Grateful acknowledgment is made to Dr. John W. Bailey, Dr. Paul B. Sears, Dr. A. P. Dachnowski-Stokes, Dr. B. S. Henry, and Mr. Henry Wirth for scientific assistance and to Mr. Sam Buck, Mr. J. P. McCutcheon, Mr. G. W. Woolard, and Spencer Bros. for courtesies and assistance in the field work.

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COMPARATIVE STRUCTURE OF THE WOOD IN THE "KNEES,"
SWOLLEN BASES, AND NORMAL TRUNKS OF THE
TUPELO GUM (*NYSSA AQUATICA* L.)

WILLIAM T. PENFOUND

(Received for publication May 16, 1933)

The river bottom swamps of the southern states are characterized by a mixed stand of the tupelo gum, *Nyssa aquatica* L., and the bald cypress, *Taxodium distichum* (L.) Richard. In logged areas the black willow, *Salix nigra* Marsh, the southern red maple, *Acer drummondii* Hook. and Arn., and the water ash, *Fraxinus caroliniana* Mill., are common associates.

Of the above trees the bald cypress is conspicuous because of its conical "knees" and buttressed bases. The southern red maple often possesses looping roots which may approximate the "knee" habit of growth, and the water ash sometimes exhibits slightly swollen bases. Among the more striking features of these swamps, however, are the greatly enlarged bases of the tupelo gum (fig. 1). These swollen bases may attain a diameter of more than three



Fig. 1. Enlarged base of the tupelo gum from which a chip for study has been removed.

times that of the normal trunk just above. The relative size of these bases appears to vary in the same direction as the hydroperiod and the depth of the water. In addition to these swollen bases, the tupelo gum often exhibits looping roots with a maximum observed height of 22 inches and a horizontal spread of 26 inches. These looping roots usually become modified to form a conspicuous "knee" or pneumatophore.

The origin of the "knee" in the tupelo gum is quite different from that of the bald cypress. In the latter the pneumatophore is developed as a knob-like dorsal protuberance of a horizontal root by local activity of the cambium. This structure attains its conical form through the activity of a cambium which is continuous over the wood but more active near the apex. It is, therefore, merely a local, but extensive, outgrowth of a root. The "knee"



Fig. 2 (left). Young "knee" of the tupelo gum.

Fig. 3 (right). Mature "knee" of the tupelo gum.

of the tupelo gum is not a local outgrowth but consists of an arched portion of a normal root. When young there is a considerable space between the root arch and the soil (fig. 2), but with secondary thickening this space is largely obliterated and a more typical "knee" develops (fig. 3).

ENVIRONMENTAL FACTORS

The area from which the material for this study was obtained is known as the Bridgedale Swamp. It is located on the Kenner Air-line highway between Kenner, La., and New Orleans, La., at a position of 30° North and 90° 8' West. The climate of the Bridgedale area is essentially coastal be-

cause of the proximity of the region to the Mississippi River, to Lakes Pontchartrain, Borgne, and Catouatche and the Gulf of Mexico (60 miles). The mean annual temperature is 69.1°F. with mean monthly temperatures of 54°F. and 82.4°F. for January and July respectively. The average annual precipitation of 58 inches is evenly distributed throughout the year. In the spring the average date for the last killing frost is February 14 and that of the first killing frost in the fall is December 7. These climatic conditions are conducive to rapid and almost continuous growth throughout a large part of the year.

The Bridgedale Swamp has been drained, but in the area from which specimens were procured, it is covered by standing water for at least six months of the year. The average water depth is about two inches, with an observed maximum of 12 inches. The average water content of the soil (based on dry weight) is near 50 per cent, and the average organic content in the first foot of soil is approximately 10 per cent. Conditions, therefore, are still favorable for the growth of swamp trees, and since the cypress has been cut in preference to the tupelo gum, the latter is the predominant tree in the area.

METHODS

Three tupelo trees with "knees," swollen bases, and normal trunks were selected for sampling. The larger trees were not used because of the difficulty of getting specimens from the unswollen part of the trunk, which begins at a point from eight to twelve feet above the soil. Of those selected, the average diameter of the swollen base was 41 inches, and that of the normal trunk was 18 inches. In this area, therefore, the swollen base is only double that of the upper portion of the trunk. In areas with a greater water depth the bases are often three to four times as thick as the trunk just above. It is probable, therefore, that the differences between the wood of the swollen bases and normal trunks as here presented are less striking than they would have been had trees from deeper swamps been used.

Duplicate chips from 2 to 4 inches in thickness, 3 to 6 inches wide, and 6 to 8 inches long were cut from the "knees," the swollen base, and the normal trunk of each tree. A portion of each was used to determine the water content of the wood, cross sections were made of other parts, and a portion of each was macerated according to Jeffrey's maceration method. In all cases only the five outer annual rings were studied. From the cross sections the average width and the average number of wood elements across the annual rings were determined. From the macerations the following determinations were made for fibers, vessels, and wood parenchyma: relative numbers, the average width, length, and wall thickness of each, and the average size of the pits in each. Since the wood parenchyma cells were similar to the wood ray cells, they were not separated from the latter in the determinations.

RESULTS AND CONCLUSIONS

As is well known, the water content of plant parts varies with the water content of the soil and the weather conditions. In the summer of 1932 the average water content of the wood of the swollen bases was 36.1 per cent and that of the normal trunks was 33.1 per cent, but in the spring of 1933 the corresponding water contents were 46.4 and 39.6 per cent, with that of the "knees" still higher (50 per cent). It will be noted also that the water content of the above parts decreases with elevation, the water content of wood of the normal trunk being significantly lower than that of the swollen base (fig. 5).

In the enlarged bases the annual rings were much wider than they were in the normal trunks (table 1). Based on the four outermost annual rings,

TABLE 1. *Average width of the four outermost annual rings and average number of wood elements across the annual rings (in parentheses) in the knees, enlarged bases, and normal trunks of the tupelo gum. Measurements in microns.*

Number of ring	Knees	Swollen bases	Normal trunks
1	560 (12)	600 (18)	370 (12)
2	1000 (25)	1100 (30)	508 (17)
3	1130 (27)	1224 (32)	650 (19)
4	1610 (32)	2100 (36)	460 (16)
Ave.	1075 (24)	1256 (29)	497 (16)

that of the "knees" was 2.2 times and that of the swollen bases was 2.5 times the width of the rings in the normal trunks. The relative number of cells along the radial axes of the annual rings was 1.5 for the "knees," 1.8 for the bases, and 1.0 for the trunks (table 1). It is thus evident that the diversity in the width of the rings in the material studied is due mainly to the difference in the number of the cells laid down.

TABLE 2. *Relative numbers of wood parenchyma cells and wood fibers (based on vessels as unity) in the knees, enlarged bases, and normal trunks of the tupelo gum*

	Knees	Enlarged bases	Normal trunks
Parenchyma	127	101	62
Fibers	10.5	7	6
Vessels	1	1	1

A study of table 2 shows that the softer wood of the "knees" and bases possessed a relatively greater number of parenchyma cells than the harder wood of the trunks. This holds true whether the relative number of parenchyma cells is calculated on the basis of the number of fibers or the number of vessels. Although the wood of the "knees" and bases is soft and parenchymatous, there are practically no intercellular spaces.

The studies on the dimensions and wall thickness of the wood components were made from well-stirred macerated tissue of the five outer annual rings. Each figure in the original tables from which figure 4 was

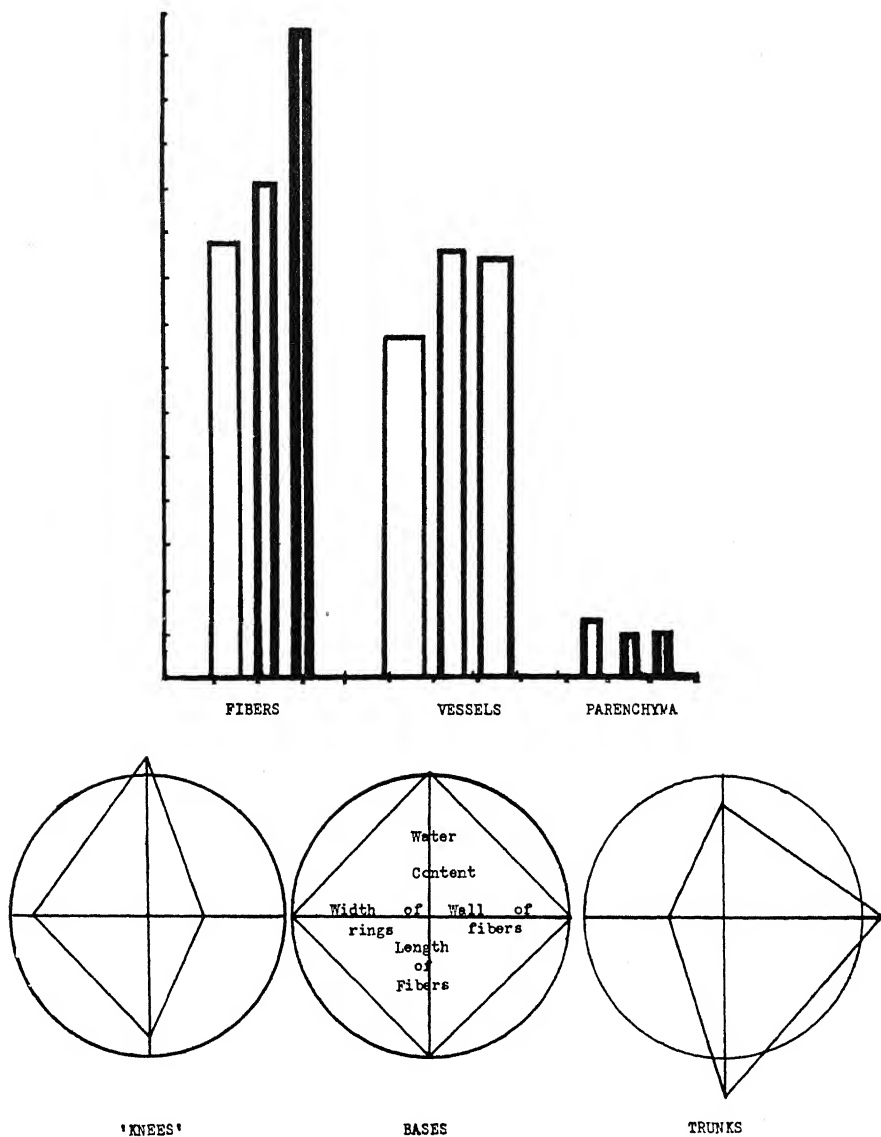


Fig. 4 (above). Average width, length, and wall thickness of fibers, vessels, and wood parenchyma cells in the "knees" (left), swollen bases, and normal trunks (right) of the tupelo gum. Width and length are shown by the width and height of the bars and wall thickness is represented by the thickness of the lines of each bar. Each space equals 100μ for width and length and 25μ in wall thickness.

Fig. 5 (below). Summary of the important features in the "knees," enlarged bases, and normal trunks of the tupelo gum. The length of the radii included within the quadrilateral indicate the relative magnitude of the features named.

constructed represents an average of measurements on 60 wood fibers, 60 vessels, and 60 wood parenchyma cells from three different trees. In addition to the averages, the maximum and minimum measurements on each component were tabulated. Since the latter have no particular bearing on our problem, the results are not presented in this paper.

The more striking differences in a comparison of the wood of the "knees," bases, and trunks were in the wood fibers. In the trunks the wood fibers were narrower, much longer, and possessed much thicker walls than those of the "knees" and bases (fig. 4). Using the measurements of the fibers of the "knees" as unity, the relative dimensions in the enlarged bases and normal trunks were as follows: width, 0.70 and 0.46; length, 1.13 and 1.46; and wall thickness, 2.3 and 2.7 respectively. The vessels were both shorter and thinner-walled in the "knees" but were not notably different in the bases and trunks. Among the more significant characteristics of the "knees" are the relatively thin, wide, and long wood parenchyma cells (fig. 4). This fact, coupled with the relatively greater number of parenchyma cells, explains the relatively spongy nature of these structures. It should also be pointed out that the wall thickness of all the wood components is greater with elevation—i.e., they were thinnest in "knees," intermediate in the bases, and thickest in the trunks (fig. 4).

Measurements of the pits of fibers, vessels, and wood parenchyma failed to reveal any important differences (table 3). The pits of the wood com-

TABLE 3. *Average width and length of the pits in the fibers, vessels, and wood parenchyma cells in the knees, swollen bases, and normal trunks of the tupelo gum. All measurements in microns.*

		Knees	Swollen bases	Normal trunk
Fiber pits	Width	6.54	5.61	5.88
	Length	9.20	8.08	8.19
Vessel pits	Width	5.04	5.14	5.67
	Length	6.12	7.77	7.77
Parenchyma pits	Width	4.83	4.48	4.83
	Length	5.56	6.09	6.33

ponents of the bases and trunks were very similar in size, but in the "knees" the pits of the vessels were somewhat smaller and those of the fibers were considerably larger than those of either the bases or trunks. The pits of all wood parenchyma cells were similar in size. It is apparent, therefore, that there is little, if any, correlation between the size of the pits and the size of the elements in which they occur.

DISCUSSION

The origin and survival value of the pneumatophores and swollen bases of woody plants have been the subject of much speculation. In saline areas the pneumatophores usually arise as peg-like outgrowths from underground roots

(Groom and Wilson, 1925). Wilson (1889) states that under submerged conditions young roots of the bald cypress often grew directly upward until they reached the surface, when they again turned and developed beneath the water. He states that similar structures occur about the base of the tupelo gum, the roots bending sharply upward to a height of six to eight inches above the surface of the water, whence they turned downward into it again. In shallow swamps the "knees" of the bald cypress are peg-like outgrowths of underground roots, whereas those of the tupelo gum are, as Wilson states, arched aerial roots which become geniculate through secondary thickening.

Wilson (1889) observed that the number and size of the "knees" of the bald cypress were determined by the height of the water and the direction of flooding. We have found that the "knees" of the cypress do not form where the hydroperiod is very short; nor do they develop in deep water where the hydroperiod is almost continuous. According to an unpublished manuscript of the United States Forest Service, the removal of cypress "knees" has little observable effect on growth. In the pneumatophores of many plants of saline areas there is a definite communication between the aerenchyma of the "knees" and that of the underground roots. But in both the bald cypress and the tupelo gum there is no well-developed aerenchyma. The value of the "knees" of these plants in gaseous exchange is, therefore, brought into question. Experiments to determine the absorption of O_2 or the release of CO_2 in the pneumatophores of bald cypress, tupelo gum, and other species are needed, therefore, to settle this controversial point.

The swollen bases of the tupelo gum and the black gum (*Nyssa biflora* Walt.) are conspicuous features of southern swamps. The average water level does not cover more than one-fourth of the enlarged bases of these species, and this general relation was also found to be true for the black ash (*Fraxinus nigra* Marsh) by Gates and Erlanson (1925). They found the bases to be less than twice the diameter of the trunk above. In both the tupelo and black gums, however, the bases are usually from two to four times the normal trunks above, especially when the hydroperiod is nearly continuous.

Gates and Erlanson (1925) found that the swollen bases were due to a great increase in the number of cells in the summer wood of each year's growth. We were unable to differentiate clearly between spring and summer wood in the "knees" and bases, but we did find that the swollen bases were due primarily to an increase in the number of cells over that of the normal trunk. The fibers and wood parenchyma cells were, however, somewhat larger in the swollen bases.

The explanation of the swollen bases is to be sought in the reactions of these species to the water which often covers a portion of the base of the trunk. As has already been stated, the size of these bases is apparently in direct relation to the length of the hydroperiod and the depth of the water. In the tupelo gum the maximum water content of the wood was greatest in the "knees," intermediate in the bases, and least in the trunks. Yapp and

Mason (1932) state that the maximal water content of leaves is found in the lowest leaves and that the minimum is reached in the youngest leaves, which were partly expanded. Other investigations support the conclusion that the water content of plant tissues decreases with the distance from the supply. Pfeffer (1903) states that maximum growth is possible only when the cells are fully turgid and that diminished stretching of the wall results in a decrease in growth. It is evident that the conditions under which the tupelo gum grows are such as to promote maximum growth and the formation of enlarged bases. But why other trees growing in the same conditions do not produce these swollen bases is at present unexplainable.

The wood of tupelo gum is spongy in the "knees," somewhat firmer in the bases, and relatively hard in the trunks. This is due to the increased wall thickness, with elevation, of all the component cells. This is especially true of the wood fibers. In addition, the wood fibers are much longer in the wood of the normal trunks. Kienholz (1931) has shown that the length of tracheids in the lodgepole pine was notably greater with elevation, especially near the bark. The greatest difference, as shown by his figures, occurred between the two- and twelve-foot levels. In view of these findings and those of other investigators, it may readily be true that the difference in length of fibers may be a function of tree height.

SUMMARY

A comparison of the wood of the "knees," enlarged bases, and normal trunks of the tupelo gum (*Nyssa aquatica* L.) showed the following differences:

1. The water content was greatest in the "knees," intermediate in the bases, and least in the trunks.
2. The wood of the "knees" was spongy, that of the swollen bases was somewhat firmer, but that of the normal trunk was relatively hard.
3. The greater size of the basal portion of the trunks was found to be due primarily to the greater number and secondarily to a greater size of the component cells of the wood.
4. The "knees" and bases possessed a relatively greater number of wood parenchyma cells than the trunks.
5. The wood fibers were progressively narrower, longer, and thicker-walled in the "knees," enlarged bases, and normal trunks.
6. The wall thickness of all the wood components increased with elevation—i.e., it was least in the "knees," intermediate in the bases, and greatest in the trunks.
7. The pits of the vessels were smaller and those of the fibers were larger in the "knees" than they were in corresponding elements of the bases and trunks. Otherwise there were no significant pit differences.

I wish to express my appreciation to Joseph McCloskey, Jr., for his help in the routine measurements on the wood components.

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ONTOGENY OF PHLOEM IN THE SUGAR BEET (*BETA VULGARIS* L.)

KATHERINE ESAU

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In connection with an investigation of the anatomical changes induced in the sugar beet by the curly-top disease, it was necessary to make a detailed study of the healthy phloem. Its structure, at maturity, was described in an earlier publication (Esau, 1933). This paper deals mainly with the developmental stages of this tissue, especially the sieve tubes. It will form the background for a study of the stages of phloem degeneration in beets affected by curly top.

The material and methods used in this work were the same as those previously described (1933). As a source of phloem tissue, the petioles proved to be better than the roots because they furnish comparatively long strands of vascular tissue, straight and free of anastomoses.

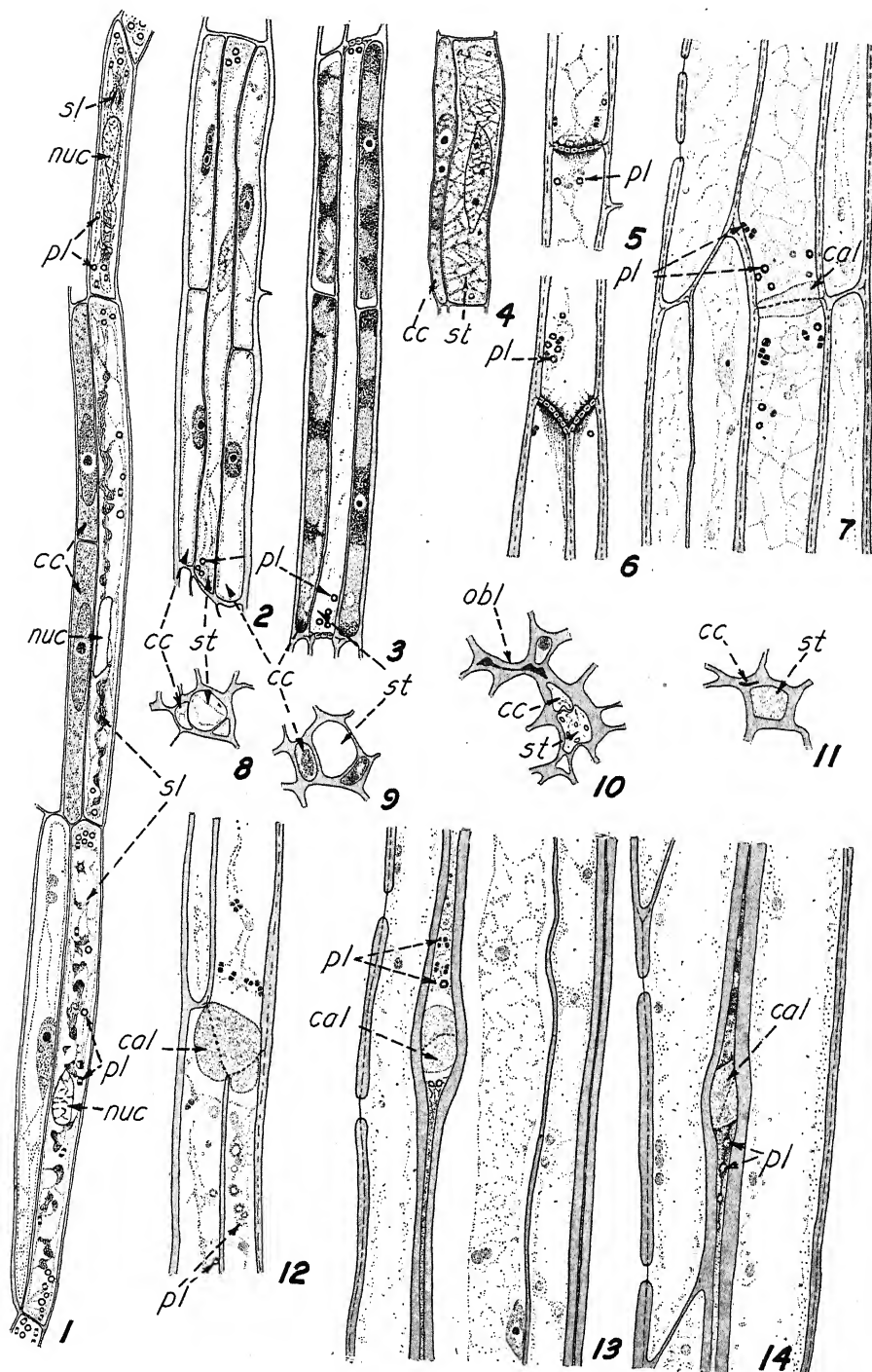
RESULTS

Young sieve tubes

The vascular tissue in beet leaves consists of collateral bundles, the phloem being located on the dorsal side (Esau, 1933, fig. 2, A). Vascular bundles arise from procambium strands. The differentiation of the primary xylem and phloem begins at two opposite ends of the bundle and progresses toward its middle. Between the two primary tissues, persisting meristematic tissue forms the cambium from which secondary xylem and phloem arise. The phloem is composed of sieve tubes, companion cells, and phloem parenchyma cells.

The sieve tube and its companion cell are derived from one mother cell

Fig. 1-14. Ontogeny of the sieve tubes. Fig. 1-7 and fig. 12-14 longitudinal, fig. 8-11 transverse sections. Fig. 1, young sieve tubes with slime bodies (*sl*), plastids (*pl*), and disintegrating nuclei (*nuc*). Fig. 2 and 8, younger, fig. 3 and 9, older sieve tubes (*st*) and companion cells (*cc*) from secondary phloem. Fig. 4, a young sieve tube (*st*) and a companion cell (*cc*) from primary phloem. Fig. 5 and 6, portions of mature sieve tubes showing the primary lamellae and secondary thickenings on the sieve plate, also slime accumulations, and plastids (*pl*). Fig. 7 and 12 show definitive callus (*cal*) on sieve plates, plastids (*pl*), and the cytoplasmic network of old sieve tubes. Fig. 10, a sieve tube (*st*) and a companion cell (*cc*) in an early stage of obliteration, and two completely crushed cells (*obl*). Fig. 11, sieve tube (*st*) and a crushed companion cell (*cc*). Fig. 13 and 14 show two stages in obliteration of a sieve tube. Fig. 4, $\times 888$; fig. 1-3 and 5-14, $\times 634$.



by longitudinal division. The two resultant nuclei are unlike, the companion cell nucleus being more granular and staining more deeply than that of the young sieve tube (fig. 4). A similar difference between the two nuclei was reported by Lecomte (1889) in the case of *Vitis*. The cytoplasm of the young sieve tubes and companion cells is vacuolate like that of the cambium. The vacuoles may be observed in fresh sections stained with neutral red, and also in paraffine preparations. This observation is in accord with the work of Bailey (1930), who has found that normal cambial initials are conspicuously vacuolated. In fixed material, vacuolation in young phloem cells is more pronounced in secondary than in primary phloem. The procambial and young primary phloem cells show numerous small vacuoles (fig. 4), whereas the cambial and young secondary phloem cells develop a few large vacuoles (fig. 2).

Slime bodies

In its further development, the sieve tube undergoes a rapid succession of complicated changes. In the cytoplasm develop the characteristic slime bodies, plastic structures shaped like irregular drops and showing, in the early stages of their development, an avidity for haematoxylin dyes. In young leaves, where cells are actively dividing, slime bodies may be readily found; in older leaves where only secondary phloem is differentiating, they seem to be absent. In fixed sections these structures may be quite discrete and partly rounded off, as in the lower cell of figure 1; or they may seem to be composed of massed threads adhering to strands of cytoplasm, as in the two upper cells in figure 1. In later stages of development, coarse, heavily stained strands appear to fill the lumina of the sieve tubes. These strands apparently result from disintegration of the slime bodies and they disappear in more mature sieve tubes. The slime bodies are inclusions of proteinaceous matter which, disintegrating, become part of the vacuolar material of mature sieve tubes (Artschwager, 1924; Crafts, 1932, 1933; Lecomte, 1889; Strasburger, 1891).

Structures resembling the slime bodies of the beet occur in young sieve tubes of many plants. Although numerous in the cucurbit sieve tubes (Crafts, 1933; Lecomte, 1889), they occur singly in certain Leguminosae (Strasburger, 1891) and Solanaceae (Artschwager, 1924; Crafts, 1933). In the cucurbit they have been described by Crafts (1933) as small drops. In *Robinia pseudoacacia* and *Wistaria* they have, according to Strasburger (1891, pp. 193, 199; plate III, fig. 4-12), an ellipsoidal or polygonal shape, attenuated to a thread at each end. Nelson (1922), who saw similar structures in virus-diseased bean and clover, interpreted them as biflagellate protozoans related to *Leptomonas*. To him, the long, sinuous slime bodies in tomato and potato appeared as trypanosoma. Later, Artschwager (1924) correctly identified them in the potato. Crafts (1932, 1933), having described in detail the origin and disintegration of slime bodies in both the cucurbit

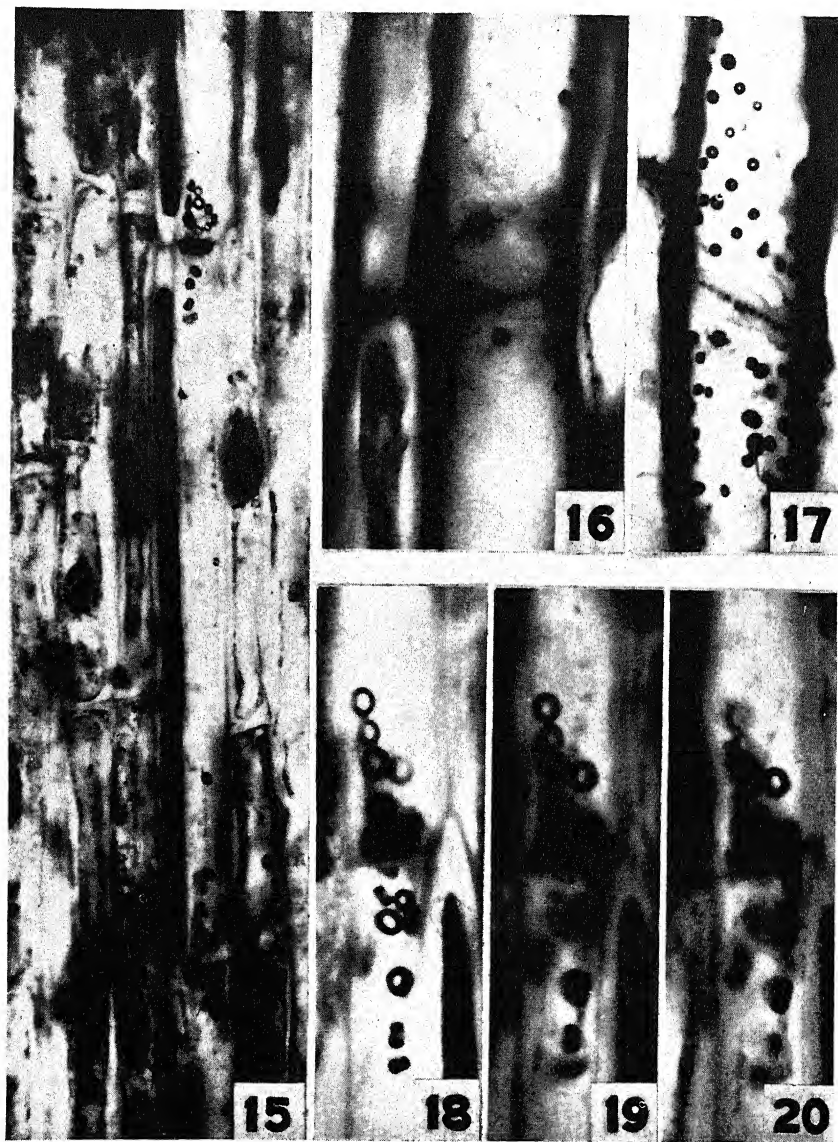


Fig. 15-20. Longitudinal sections of sieve tubes. Fig. 15, a sieve tube element with plastids near its two sieve plates ($\times 1000$). Fig. 16 and 17 show sieve plates covered with definitive callus, and plastids near the plates ($\times 1200$). Fig. 18-20, sieve-tube plastids in different positions ($\times 2200$).

and the potato, has shown that they originate in the cytoplasm, pass through the various stages of development and disintegration in contact with it, and later disappear as discrete bodies, becoming part of the colloidal material suspended in the sieve-tube vacuole.

On treatment with killing agents, the proteinaceous vacuolar material has been shown by certain workers to coagulate and to accumulate near the sieve plates (Crafts, 1932, 1933; Schmidt, 1917). In fixed sections of beet phloem such accumulations occur in sieve tubes in which the slime bodies have ceased to be discrete. This condition is shown in figures 5 and 6. Fine strands of parietal cytoplasm appear to be continuous with the slimy material on the plate.

Sieve-tube plastids

The most characteristic and permanent cytoplasmic structures of sieve tubes are the plastids. Soon after the completion of the division that gives rise to sieve tube and companion cell, they become visible (fig. 2, 4). These plastids have a characteristic shape—that of a disc whose margin stains heavily while the center remains light (fig. 1-7, 15, 17-20). They also resemble rings, but the clear area in the center is not a perforation. When lying on edge, they appear like two hemispheres separated from each other by a faintly stained area (fig. 1, 5, 7, 12, 18). Sometimes they resemble thick rings (fig. 17); in other cases they have a large clear central area (fig. 18-20). This latter type is particularly common in younger leaves. Occasionally the plastids appear swollen, the clear central area being much reduced. In very young sieve tubes they are somewhat smaller than in old cells. Aside from these variations, however, they are fairly constant in shape and size.

In youngest sieve tubes the plastids are scattered throughout the cytoplasm of the cell (fig. 1), but in slightly older elements they are found, in prepared sections, near the sieve plates (fig. 2, 3, 15). They do not show Brownian movement in living beet sieve tubes and have not been observed in the vacuole. According to Crafts (1933), in the potato the plastids of old sieve tubes move into the vacuole.

Figure 15 shows a sieve-tube element in fixed phloem tissue of a young leaf. The plastids have accumulated on both sides of the sieve plates, which are covered with slime. Strands of cytoplasm are perceptible in the sieve tube, which is flanked by a very darkly stained companion cell. On the right of the sieve tube are cambium cells; on the left, phloem parenchyma. Above the plate, in figure 18, the plastids are pictured lying on their broad sides; below, four of them are lying on edge and appear like bi-polar bodies. The third plastid from below in figures 18, 19, and 20 is seen in three positions: on its broad side in 18; on edge in 20; and in an intermediate position in 19. Figure 17 shows sieve-tube plastids near a callused plate in an old sieve tube of the secondary phloem.

After the tube has been partly crushed in the process of obliteration (fig.

14), or somewhat earlier (fig. 12), the plastids disintegrate. First, they appear to swell; then they lose their sharp outline and their staining capacity; and finally they dissolve.

In the beet, the plastids of the sieve tubes are smaller than those of other cells. Their characteristic shape and their avidity for cytoplasmic stains make it remarkably easy to recognize the sieve tubes in beet phloem. These plastids are present in all parts of the plant and probably in every sieve-tube element.

Sieve-tube plastids of the beet have been seen by other workers. Art-schwager (1926) shows these structures in a photograph of a sieve tube (p. 153, fig. 7B) but does not describe them. Smith and Boncquet (1915), who saw the plastids in beets affected by curly top, first held them to be the causal agent in curly-top infection and interpreted them as *Bacterium dianthi*. Later they discovered the same structures in plants free from curly top, but continued to regard them as organisms. These writers described ring and bi-polar bodies, realizing, however, that these were the same structures seen in two different positions.

The sieve-tube plastids and their relation to carbohydrates occurring in sieve tubes have interested investigators for many years. According to Schmidt (1917), Arthur Meyer in 1883 found colorless "trophoplasts," containing small starch grains, in the sieve tubes of certain plants. Strasburger (1891, p. 69), noticing small grains in the cytoplasm of Gymnosperm sieve tubes, considered them to be leucoplasts which produce starch, the latter also occurring in the form of small grains. In *Vitis* (Strasburger, 1891, p. 249) he saw starch formation in the leucoplasts of sieve tubes. Crafts (1933) described plastids accumulating "condensed carbohydrate" in sieve tubes of the potato. Briosi (1873) reported the presence of starch as small grains in sieve tubes of 129 out of 146 plants. As these grains appeared much smaller than those in other cells, he thought that they could pass through the pores of the sieve plates. Lecomte (1889) rejected this suggestion.

The sieve-tube plastids of Gymnosperms have been described by Strasburger (1891, p. 69) as being highly refractive and staining brown with iodine. Similar staining reaction was observed in sieve-tube plastids in the beet (Esau, 1933). The carbohydrate, however, which is produced in the sieve tubes of certain plants, stains pink or wine red (Strasburger, 1891, pp. 69, 290; Crafts, 1933; Lecomte, 1889; Schmidt, 1917); it has not been observed in connection with the sieve-tube plastids in the beet.

Maturation and obliteration of the sieve tubes

According to Strasburger (1891, pp. 194, 289, 291) and others, the nucleus usually disappears as the sieve tube approaches maturity. Lecomte (1889), however, does not consider this to be a general phenomenon: he states that the nucleus, as a discrete body, is absent in some plants, but is retained in

others in fully developed sieve tubes. Schmidt (1917) asserts that a functioning sieve tube is a normal cell, since it contains both cytoplasm and a nucleus.

In the beet the nucleus disintegrates during the development of the sieve tubes. It is not very prominent even in young cells. It stains faintly and has vacuoles (fig. 2, 4), which later enlarge, while its ability to accumulate stain decreases, as is shown in the lower cell in figure 1. Finally the nucleus appears to have one large central vacuole and no inner structure—the condition represented in the middle cell in figure 1. Sometimes it appears to swell before disintegration. When the slime bodies have disintegrated, the nucleus also disappears as a discrete body.

The walls of the sieve tubes thicken perceptibly in their early ontogeny—a phenomenon clearly observed in fresh sections, but less pronounced in the dehydrated paraffine material. The thick-walled sieve tubes are particularly conspicuous in the primary phloem.

Many investigators have noted the thickness and the peculiar luster of the sieve-tube walls, which, however, have been found not to differ chemically from other cellulose walls (Schmidt, 1917). Crafts (1931) showed that phloem walls are thick because of hydration.

The areas where sieve perforations develop become perceptible at about the time when slime bodies are formed (fig. 1). While the latter are disintegrating, the sieve plate is thickened by deposition of secondary lamellae, and the pores become distinct (fig. 5, 6). At this stage the sieve tube is regarded as mature: it has attained its full length, the cytoplasm has become reduced to a thin parietal layer, the nuclei and slime bodies have disintegrated, and the cytoplasmic connections (Crafts, 1932; Schmidt, 1917) through the sieve plates have become quite evident.

The mature state imperceptibly passes over into senility. The sieve plate is modified by callus development. According to certain workers (Strasburger, 1891, p. 288; Crafts, 1932; Hill, 1908; Schmidt, 1917), the callus first lines the perforations and later covers also both surfaces of the plate. It continues to develop until it forms a large mass of definitive callus, within which the primary lamella of the sieve plate appears, in longitudinal sections, as a broken line (fig. 7, 12, 17).

The presence of definitive callus indicates the approaching obliteration of the sieve tube. This process involves complete loss of contents and crushing of the sieve tubes by phloem parenchyma cells. Obliteration in beet phloem has been partly described in a previous paper (Esau, 1933). Figures 7 and 12 show sieve tubes approaching obliteration. They depict, in fixed sections, the definitive callus, the middle lamella of the sieve plate within the callus, the plastids near the plate, and the cytoplasm as a fine network within the sieve tube. In figure 12 some of the plastids are disintegrating. Parts of similar sieve tubes are shown in figures 16 and 17. When the sieve tubes are crushed, the definitive callus does not disappear suddenly. Though the walls

of phloem parenchyma obliterate the lumen of the sieve tube, they are kept apart in the sieve plate region by the mass of definitive callus, as shown in figures 13 and 14. Later the callus is probably absorbed, for it disappears, along with all traces of the sieve plate. In fresh sections the walls of the sieve tubes seem to thicken in early stages of obliteration, probably through hydration, but this phenomenon has not been recognized in fixed material.

Companion cells

The cell derived from the same mother cell as the sieve tube may develop directly into a companion cell (fig. 4) or may divide transversely, giving rise to two companion cells (fig. 2, 3). The latter condition is characteristic of the secondary phloem. The sieve-tube mother cell may undergo more than one longitudinal division, thus giving rise to one sieve-tube element and two companion cells, as seen in transverse section (fig. 8, 9), or one sieve-tube element and four companion cells when viewed in a longitudinal section (fig. 2, 3). The longitudinal division of the sieve-tube mother cell is unequal, inasmuch as the cell which will differentiate into a sieve tube is larger than that which will become a companion cell. Usually the companion cells are cut off in the corners of the mother cell in secondary phloem (fig. 8, 9).

The companion cell, as it develops, increases in staining capacity and, in fresh sections, shows a pronounced tendency to accumulate neutral red. These characteristics help to distinguish companion from phloem parenchyma cells. Figures 2 and 8 show younger, and figures 3 and 9 older companion cells from secondary phloem. The cytoplasm of companion cells is vacuolate and contains chloroplasts, readily seen in fresh sections but often obscured by the deeply stained cytoplasm in prepared sections. The long, narrow nucleus stains deeply. Its limits are sometimes not readily recognized because of densely stained cytoplasm (fig. 3).

As already mentioned (Esau, 1933), companion cells are crushed with the sieve tubes. The stages in their obliteration have been clearly observed in this study. Before being crushed, the companion cell appears narrowed, the cytoplasm and nucleus disintegrate, and finally the lumen is obliterated. In figure 10 one sieve tube and its companion cell have been partly crushed, the other two completely obliterated. In figure 11 the sieve tube has not yet been crushed in the sieve-plate region, whereas the lumen of the companion cell has been obliterated.

Phloem parenchyma

The phloem parenchyma cells are wider and longer than the companion cells, and have oblique or transverse end walls. They contain nuclei, vacuolate cytoplasm, and chloroplasts. Instead of being obliterated, they enlarge and divide in the older phloem region, crushing thereby the functionless sieve tubes and companion cells.

In the sugar beet, phloem parenchyma cells have a remarkable character-

istic of readily becoming meristematic, not only when influenced by injury, but also in the normal development of the plant (Esau, 1933). They, as well as the pericycle cells, form the secondary cambium, which gives rise to the supernumerary layers of vascular tissue (Artschwager, 1926). Evidently, then, they are less specialized than the sieve tube and companion cells that play no part in the cambium formation.

The phloem as a whole

As seen in transverse sections, sieve tubes and companion cells form groups, surrounded by phloem parenchyma cells, which are proportionately more numerous in the primary phloem than in the secondary. Figures 21 to 26 show in cross section the development of primary phloem in a leaf bundle. In figure 21 the procambium strand may be recognized by the small size of its cells. In the bundles in figures 22 and 23 one layer of cells separates the first primary sieve tubes from the starch-sheath mother cells. The cells in the layer adjacent to the starch sheath have been interpreted as pericycle (Esau, 1933). Their subsequent behavior appears not to differ from that of phloem parenchyma cells. In small lateral bundles sieve tubes and companion cells may lie next to the starch sheath, but in the larger bundles there is usually one layer of pericycle cells. In the very large bundles, especially at the base of the petiole, more than one layer of cells occur between the first sieve tubes and the starch sheath (Esau, 1933).

The following sieve tubes develop in deeper layers of the procambium strand (fig. 24). Few sieve tubes arise directly from the procambium, because definite tangential divisions, indicating formation of cambium, occur very early in the development of a vascular bundle (fig. 23, 24).

New sieve tubes and companion cells are being added to the phloem throughout the growth of the bundle, and old ones are crushed in the process of obliteration. Figure 25 shows at *obl* the first crushed sieve tube and companion cell. In figure 26 appear three obliterated groups, the third one still showing some contents in the partly crushed cells. The phloem parenchyma cells near this group have enlarged and divided. Between the parenchyma cells of the old phloem appear, like wall thickenings, crushed phloem elements. Remnants of the cell lumina in the form of narrow slits may or may not be present in these areas. As described elsewhere (Esau, 1933), the pericycle and the region in which sieve tubes and companion cells have become obliterated form in a beet leaf the bundle cap of a vascular bundle. Such a structure is shown in figure 26. The pericycle and old phloem parenchyma cells have enlarged; their walls have thickened. The lumina of obliterated elements are still evident.

Obliteration begins in very young leaves. Figure 27 represents a transverse section through the epicotyl of a young beet plant at the level of the growing point. It does not show the two cotyledons. The outermost leaves,

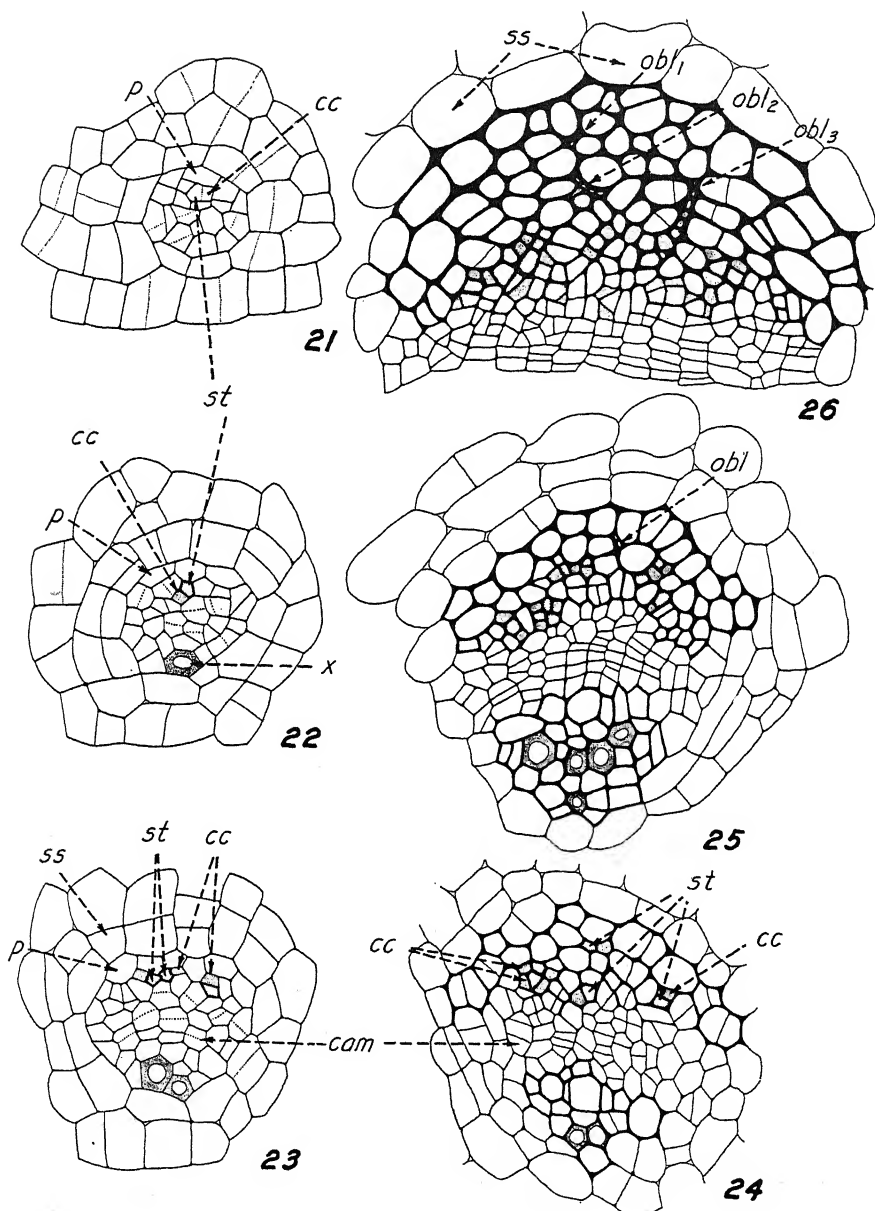


Fig. 21-26. Successive stages of phloem development in a vascular bundle of a leaf. Transverse sections. The companion cells are stippled throughout, the sieve tubes only along their margins. *cam*, cambium; *cc*, companion cell; *obl*, obliterated phloem elements; *obl* 1, 2 and 3 in fig. 26, three groups of cells in different stages of obliteration; *p*, pericycle; *ss*, starch sheath; *st*, sieve tube; *x*, xylem. Fig. 21-23, $\times 569$; fig. 24-26, $\times 364$.

numbers 1 and 2, are the first two foliage leaves that stand opposite each other and alternate with the pair of cotyledons. The following leaves are arranged alternately, with a divergence of five-thirteenths. The fifth leaf of this plant was 1.5 cm. in length.

In the following the phloem of the median bundles is described. Obviously each leaf in the section in figure 27 was cut at a slightly higher level

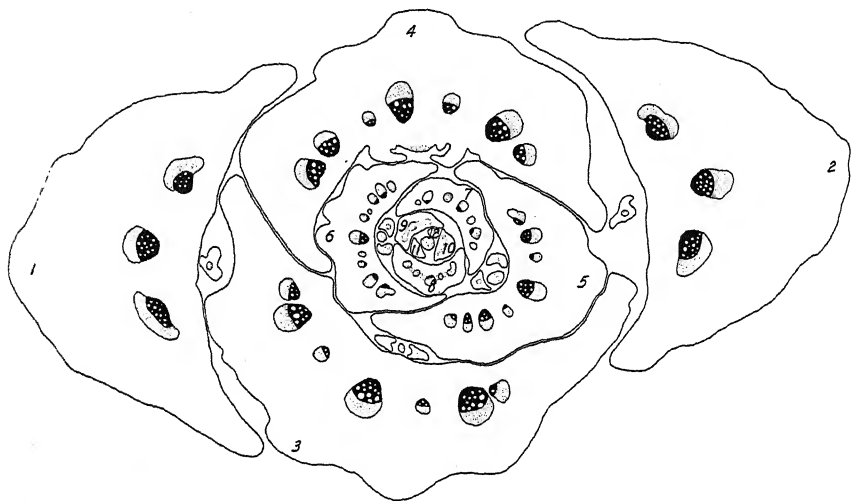


Fig. 27. Transverse section through the epicotyl of a young beet plant. In the center is the growing point, 1 is the oldest, 12 the youngest leaf.

than the succeeding younger leaf. Consequently these leaves illustrate only approximately the development of the phloem in a median bundle.

The youngest leaf, number 12, was wholly composed of meristematic cells. Leaf 11 showed enlargement of cortical cells and formation of a procambium strand. One sieve tube had differentiated in the median bundle of leaf 10; two in that of leaf 9. Leaf 8 showed five sieve tubes, of which one, the first, was slightly crushed. The phloem of the median bundle of 7 was similar to that of 8, except that the crushing of the first sieve tube had progressed farther and its companion cell had been obliterated. Leaf 6 showed six intact sieve tubes, two partly crushed, and two completely obliterated. Leaf 5 had four obliteration areas, two sieve tubes in the functioning phloem, and three in early stages of obliteration. Leaf 4 showed five obliteration areas, two sieve tubes partly crushed, two old sieve tubes, and eighteen sieve tubes in the functioning phloem. In leaf 3 the exact count of obliteration areas was rather difficult, because the traces of first sieve tubes completely disappeared. There were about ten obliteration areas, each apparently involving more than one sieve tube, and approximately twenty-four intact sieve tubes. Leaves 1 and 2 showed a situation similar to that in leaf 3. Being the first foliage

leaves, they do not become very large, nor do their bundles attain the size of those in the subsequent leaves.

Cambial activity begins early in beet leaves. It was initiated, in leaf 8 in figure 4, at the time of rapid elongation. Consequently many cells cut off from the cambium are elongating during the progress of differentiation, so that mature cells are considerably longer than cambium cells from which they were derived.

Obliteration also begins some time before the leaf is fully expanded, involving not only primary but also secondary sieve tubes and companion cells. The first groups of cells that undergo simultaneous obliteration are small; the later are larger (fig. 26). In consequence of obliteration, the bundle cap in old bundles grows large, while the layer of functioning phloem remains comparatively narrow.

This study indicates that the sieve tubes and companion cells are comparatively short lived in actively growing beet leaves. Without interruption they pass through the early stages of ontogeny, reach maturity, become senile, and are obliterated. New sieve tubes and companion cells replace old ones. One might think that the continued renewal of the essential elements plays a rôle in the function of beet phloem.

Priestley (1930) suggests that the downward movement of organic materials is closely associated with the growth processes and that "the structural features of the adult sieve tube may rather be analogous to those features in a dry river bed which supply evidence that it was once a channel along which a rapid current flowed." Most phloem workers, however, consider that the sieve tube performs its specific function at the height of its development. From either point of view, the sieve tube is certainly a highly specialized cell, probably prepared by its complex ontogeny for a special function. Though to define that function is beyond the scope of this investigation, the continuous differentiation of these elements in the sugar beet seems especially significant.

SUMMARY

The sieve tube in the sugar beet is a highly specialized cell with a complex development. During its ontogeny, plastids and slime bodies develop; the nucleus and slime bodies disintegrate; the walls thicken; and the cytoplasm decreases in amount. Meanwhile, sieve plates develop, definitive callus is formed, and finally the sieve tube is crushed by phloem parenchyma cells in the process of obliteration.

The companion cell has dense cytoplasm, a prominent nucleus, and chloroplasts. It is closely associated with the sieve tube inasmuch as both are derived from the same mother cell, occur always side by side, and are obliterated at the same time.

Cells of the phloem parenchyma are, like other parenchyma cells, little specialized. By renewed meristematic activity they may function in cambium

initiation, or by simple division they may add to the phloem or bundle cap tissue. Their enlargement helps to obliterate sieve tubes and companion cells.

Cambial activity is initiated in very young leaves and continues throughout the growth period. Obliteration of sieve tubes and companion cells also starts early and continues while new phloem elements are formed.

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THE TOXICITY OF NORMAL ALIPHATIC ALCOHOLS. I.

R. DARNLEY GIBBS

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It has been generally assumed for many years that the normal aliphatic alcohols obey Richardson's law (1869, 1883), toxicity increasing regularly with the length of the carbon chain. Further, it has been thought that the toxicity is trebled for each CH_2 group—giving the so-called Traube series (1891, 1904). Thus 11 per cent ethyl alcohol ($\text{CH}_3\text{CH}_2\text{OH}$) is about as toxic as 3.7 per cent propyl alcohol ($\text{CH}_3\text{CH}_2\text{CH}_2\text{OH}$) or 1.2 per cent butyl alcohol ($\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{OH}$). Few have experimented with more than the first five of the series.

Macht and Meyer, however, in a recent paper (1933) have published the results of work with eighteen of these alcohols, using as "biological" material seedlings of *Lupinus albus*. They placed seedlings in dilute solutions—usually 1 part of the alcohol in 10,000 parts of Shive's (1915) solution—and compared the rate of growth with that in Shive's medium alone. From these results they calculated the "phytotoxic index" for each alcohol—this being the rate of growth expressed as a percentage of the growth in Shive's solution.

The values they obtained are plotted as the broken line in figure 1. The first five (methyl to amyl) and the ninth and tenth (nonyl and decyl) appear to follow the Richardson law, while the sixth, seventh, and eighth and the members above the tenth are much less toxic than they "should" be. The slight toxicity of the alcohols above decyl can be explained in terms of solubility, for all the higher members of the series are practically insoluble in water. The low toxicities of hexyl, heptyl, and octyl are less understandable, but the following possibilities are evident: (a) *Lupinus albus* is peculiar in its relative insensibility to hexyl, heptyl, and octyl alcohols. (b) *Lupinus albus* and related plants show this characteristic. (c) All plants (and perhaps all animals) are alike in this respect. (d) The peculiar results are due to impure alcohols or to other errors and do *not* represent the true state of affairs, the alcohols really obeying the Richardson law without deviations other than those due to insolubility.

In the present investigation we have used thirteen normal alcohols—methyl to dodecyl, inclusive, and cetyl (hexadecyl). While most of these were the purest obtainable from the Eastman Kodak Co., the ethyl alcohol was ordinary laboratory "absolute," the hexyl came from two sources (E. K. Co. and Schuchardt), the heptyl from Kahlbaum, the decyl and undecyl from Compagnie Parento, and the hexadecyl from an unknown source.¹

¹ I have to thank Dr. C. F. H. Allen for samples of several of these alcohols.

The "biological" material consisted of seedlings of *Lupinus polyphyllus alba*, *Pisum sativum* ("Alaska"), *Cucurbita Pepo* (pumpkin), and *Helianthus annuus* (sunflower), as well as suspensions of yeast (freshly prepared from Fleischmann yeast-cakes).

Macht and Meyer used solutions of the alcohols in Shive's medium, and we employed the same mixture in our early experiments. We found, however, that the seedlings behaved in exactly similar fashion when the alcohols were dissolved in tap-water, and this was used in all our later experiments.

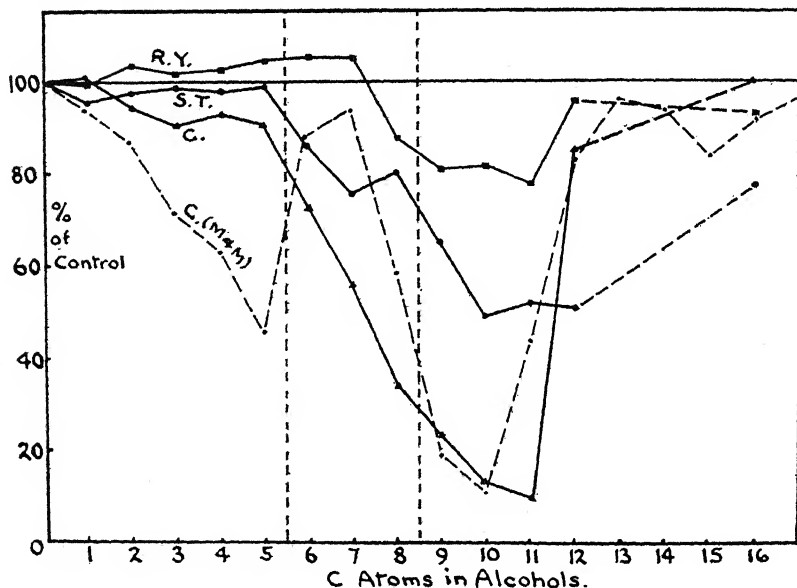


Fig. 1. Growth of seedlings and respiration of yeast in and surface tension of dilute solutions of normal aliphatic alcohols. Methyl to octyl 1 part in 10,000 nonyl to cetyl (hexadecyl) as saturated solutions (less than 1 part in 10,000). G., average growth of four species used in the present investigation. S. T., surface tension to air of alcohol solutions. R. Y., respiration of yeast. Broken line (G., M and M), growth of seedlings of *Lupinus albus* (Macht and Meyer, 1933). Control, 100 in each case.

We have already referred to the relative insolubility of the higher alcohols. Macht and Meyer report using solutions of one part in 10,000 of alcohols up to and including tetradecyl. We have been unable to make such solutions. Even with prolonged boiling (in an all-glass refluxing apparatus), the alcohols from octyl up could not be obtained in such high concentrations. Unfortunately there is little information available as to the solubilities of the alcohols. The only data that we could find for any above amyl (solubility about 2.7 per cent at 22°C.) were due to Motylewski (1904). He gives the solubilities of heptyl and octyl as 0.33 and 0.087 per cent respectively. An examination of his figures suggests that these values are due to an error in his calculations and that his results should read 0.0033 and 0.00087 per cent

(i.e., about 1 in 30,000 and 1 in 100,000). Our own observations would put the solubility of heptyl at something *over* 1 in 10,000 and that of octyl at *less* than 1 in 10,000. The alcohols above octyl are certainly much less soluble than this.

The results of rather more than a dozen experiments are summarized in figures 1 and 2.

It will be seen that the curves obtained from the four species employed

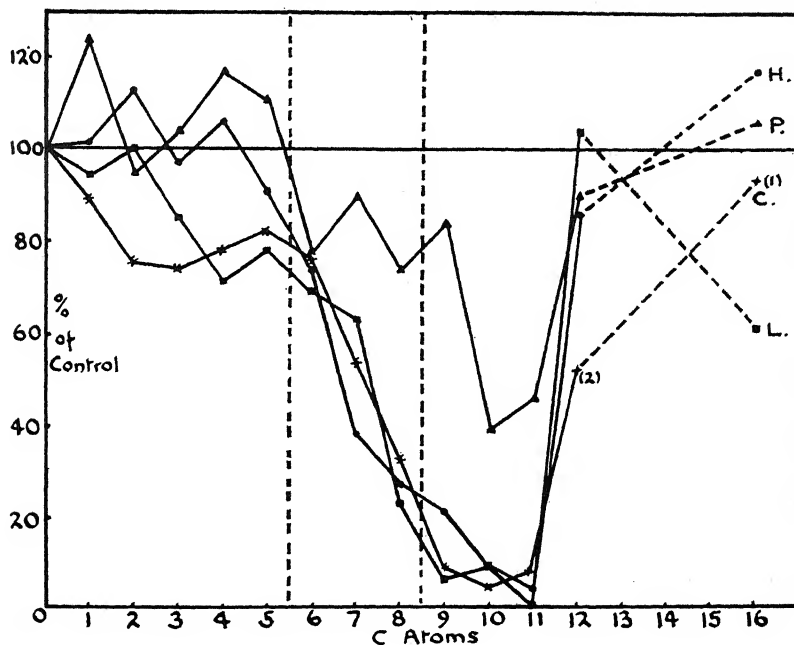


Fig. 2. Growth of the roots of seedlings in dilute solutions of normal aliphatic alcohols. L., *Lupinus polyphyllus alba* (3 experiments). P., *Pisum sativum* (2 experiments). C., *Cucurbita Pepo* (3 experiments). H., *Helianthus annuus* (5 experiments).

are very similar (fig. 2). In general, toxicity increases up to undecyl alcohol, while dodecyl and cetyl (hexadecyl) alcohols are practically innocuous (due to insolubility). There is absolutely no indication of the low toxicities of hexyl, heptyl, and octyl alcohols noted by Macht and Meyer for *Lupinus albus*.

It should be emphasized that the results given include *all* experiments. Many of our early experiments were carried out with small batches of seedlings and with imperfect technique. In particular, we worked, in those early experiments, with solutions containing droplets of undissolved alcohol. The curves obtained in later experiments, however (in which our material was pipetted off from below the floating droplets of undissolved alcohol), were smoother but not substantially different from those secured in these early attempts.

The results for *Pisum sativum* appear to differ markedly from those for other seedlings, but this is traceable to several facts. Only two experiments were carried out—one a very unsatisfactory early attempt with few seedlings. Growth in *Pisum* was relatively slow, so that small errors of measurement are greatly magnified, while the slight growth that took place (as in all seedlings) even in the most toxic solutions (and apparently before the alcohols take effect) appears disproportionately large. The average for all seedlings (*G*, fig. 1) gives a much better idea of the results obtained in later experiments but does not bring out a feature which may be significant. We have obtained some indication, in our later, more reliable tests, of an actual stimulation of growth by the lower members of the series. This will receive further consideration in a later paper—but see the results from yeast described below.

Czapek's observations on surface tension and toxicity (1911) led him to conclude that the surface tension against air of a solution of critical toxicity is 0.68 (that of water being taken as unity). His conclusions have been criticized by a number of workers, and notably by Stiles and Jørgensen (1917), who showed, employing rate of exosmosis of solutes from cells as a measure of toxicity, that "... rate of exosmosis produced by a solution is not a function of its surface tension alone."

We have measured the surface tension against air of all our solutions—using a Cenco-DuNoüy tensiometer—and the results are recorded in figure 1 (curve *S. T.*). It will be seen that the surface tensions of the first five solutions are substantially equal to the surface tension of water, and that there is a more or less steady decrease in tension of the solutions up to that of decyl alcohol. Again the curve is an average of all experiments, and the presence of drops of undissolved alcohol in our early solutions led to somewhat low results. Thus the surface tensions of decyl, undecyl, dodecyl, and hexadecyl (which appear in figure 1 as 0.49, 0.52, 0.51, and 0.77, respectively) were found, in solutions free of undissolved alcohol, to be 0.56, 0.53, 0.64, and 0.99.

Actually the only solutions that killed the roots of seedlings in twenty-four hours were those of nonyl, decyl, and undecyl alcohols with surface tensions of 0.69, 0.56, and 0.53. These would seem to fall in with Czapek's rule, but dodecyl alcohol, with a surface tension of 0.64, is practically non-toxic (phyto-toxic index in later experiments 95), which is *not* in line. With this exception, it may be said that surface tension and toxicity are closely correlated.

It was felt that respiration of yeast, as measured by CO_2 output, might well be similarly affected by alcohols. We have carried out a number of experiments, using the same solutions of alcohols and the following simple technique.

Ordinary respiration tubes (that had been carefully standardized by a number of control experiments) were used and 1 gm. of cane-sugar was placed in each; then 20 cc. of the appropriate alcohol solution were added and 1 cc.

of a freshly prepared yeast suspension. The time required for the evolution of 1 cc. of CO_2 was taken as a measure of the activity of the yeast. The results (expressed as percentages of the respiration of a control) are given in figure 1 (curve R. Y.). The curve is not unlike the curves for seedling growth and surface tension but differs in one important respect. There is a strong indication of an actual stimulatory effect by the early members of the series, the effect extending even to heptyl alcohol. The mathematical probability that this indication of stimulation is significant is about 20:1 for the first seven alcohols considered as a group and even higher for amyl, hexyl, and heptyl taken together. While our experiments are not yet extensive enough to justify a dogmatic statement, we feel that the stimulation is an actual fact and are carrying out further experiments to check our figures.

It is clear that we have found no indication of the "irregular" action of solutions of hexyl, heptyl, and octyl alcohols on growth of the roots of seedlings as reported by Macht and Meyer. Their results, if free from errors due to impurity of alcohols, would seem to apply to *Lupinus albus* alone and not to nearly related species such as *Lupinus polyphyllus alba* and *Pisum sativum*, nor to *Helianthus annuus*, *Cucurbita Pepo*, and yeast. It is obviously desirable to check up on *Lupinus albus* as well, and this we hope to do in the near future.

The experiments described above deal with solutions containing 1 part in 10,000 or less of the alcohols studied. It is worth while to investigate the action of more concentrated solutions with a view to establishing limiting toxicities. This has been done, using other methods and materials, by Kovář (1930, imbibition), Munch and Schwartze (1925, rabbits), Regnard (1889, yeast), and Stiles and Stirk (1931a, exosmosis of solutes from potato tuber). Preliminary experiments with yeast have yielded results in fairly close agreement with those of Regnard (1889). It is proposed to deal with this and other work in a later paper.

SUMMARY

1. The relative toxicities of dilute solutions of thirteen normal aliphatic alcohols (as measured by growth of roots of *Lupinus polyphyllus alba*, *Pisum sativum*, *Cucurbita Pepo*, and *Helianthus annuus*) have been determined.

2. The results are not in agreement with those of Macht and Meyer for *Lupinus albus*.

3. Surface tension and toxicity are closely correlated for the most part, but dodecyl alcohol (with surface tension 0.64 relative to water) is practically non-toxic.

4. Respiration of yeast in the same alcohol solutions has been studied. There are indications of stimulation by early members of the alcohol series, marked depression by solutions of octyl, nonyl, decyl, and undecyl alcohols, and practically no effect in the case of dodecyl and hexadecyl alcohols.

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THE CYTOLOGY OF THE INTERSEXUAL FLOWERS OF *MERCURIALIS ANNUA*—A MORPHOGENETIC STUDY

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The embryologist has studied the fate of the cells that arise from the fertilized egg. From a relatively simple organization he has witnessed a gradual increase in complexity which results in differentiation of cells, tissues, organs, and finally in an integrated organism that bears no resemblance to the fertilized egg from which it has arisen. He has watched mounting complexity go on in an orderly fashion just as if an unseen guiding principle directed every movement. The observations of the embryologist of today are amplifications of those of Wolff, who in 1759 gave back to the world an epigenetic concept of development which had been denied it ever since Aristotelian writings were branded as pagan. The preformationists of Wolff's time, and before, had contributed nothing to the solution of the problem of growth and development. Their contention that the egg or sperm contained a miniature replica of the mature individual was merely a dialectic approach to morphogenetic causes. When Wolff showed that a chick embryo achieves complexity by the gradual addition of tissues and organs, and that the final result, the fully formed chick, was not a mirror image of the germ from which it developed, epigenesis replaced preformationism. Wolff's objective research methods replaced groundless, metaphysical conjectures.

The revolutionary nature of Wolff's findings became even more apparent when the genetic continuity of the cells that make up the embryo was established. The concept of the cell as the unit of life became the measuring rod in biological surveys. The egg is a cell; the sperm is a cell. From a union of the two a large number of cells are born, and as the cell population increases, the complexity of the growing embryo also increases. Whether we retrace the steps from complexity to the fertilized egg stage or follow the paths laid out by the cells as they increase in number and complexity, there is no break in the long chain of events between the initiation of morphogenetic processes and the end product, the fully formed adult.

The student of morphogenesis has tried to understand and analyze the molding forces. The preformationist was not concerned with formative forces. To the question, "How did it come to be?" the preformationist Albrecht von Haller (Thomson, 1913) answered, "Es giebt kein Werden." Wolff's *nisus formativus*, Driesch's *entelechy*, Bergson's *élan vital* try to establish guiding principles—metaphysical preformationist architectures—after which material things (living matter) are patterned. The march of

events in science has decreed otherwise. The physical and the biological sciences became integrated. Protoplasm, a semi-liquid substance, possesses attributes which only living matter possesses; yet it obeys physical and chemical laws as non-living substances do. Nehemiah Grew's concept of the lace-work arrangement of cells does not seem fantastic in the light of Plateau's surface tension studies with soap bubbles. Hofmeister and Sachs saw in the planes of divisions of plant cells a recognition of geometric relations. Sachs saw in the dividing groups of cells planes of cleavage describing two sets of confocal parabolas. The rôles of temperature, light, chemicals, gravity, and other physical forces in shaping biological expression were recognized. It must not be forgotten that with the publication of Darwin's "Origin of Species" the last stronghold of special creation was taken so that the mechanistic concept of life prevailed and activated scientific thought. Given an egg of a certain genetic constitution, the form taken on by the individual arising from that egg will be the resultant of the forces of heredity and the physical and chemical forces surrounding the developing egg. The microscope revealed no preformed organization in the germ of the egg nor in the sperm.

How then could complexity of organization be explained? The physical and chemical forces act equally upon frog eggs, upon chick eggs, upon mammalian eggs; yet the results are different. The answer to that question was micellae, gemmules, pangens, physiological units. Certainly these morphological units tell no more than did the *nisus formativus*, the *entelechy*, the *élan vital*. The scientist thought that he had escaped preformationist contamination but he was mistaken.

It is a curious bit of irony to know that the very means that accomplished the revolution in embryology and emancipated it from the preformationism of the seventeenth and the eighteenth centuries served also as the weapon of the counter-revolution which was to force the world back into the beliefs which their progenitors had abandoned. His, Roux, and Whitman, to mention but three outstanding embryologists, advanced the pre-localization concept. They found organ-forming germ regions in the unfertilized egg. They assumed that whatever differentiation goes on as a result of cleavage is foreshadowed in the cytoplasm of the egg. With but little modification Bonnet's *emboîtement* theory was recalled from exile. This time it had the sanction of science.

A new science began to clamor for attention and received it. Cytology, by a brilliant series of sorties, won the attention of the investigators. The spectacular manoeuvres of the nucleus in bringing about division of itself into two left no doubt in the minds of scientists about its importance in hereditary transmissions. Preformationism shifted its position from the cytoplasm of the egg to that of the nucleus of the egg and sperm. Weismann's biophores, determinants, *ids*, and *idants* found an abiding place in the chromosomes, and thus a new link was forged and added to the preformationist chain.

Weismannism was saved from becoming an historical landmark only by another fortuitous circumstance—the rediscovery of Mendel's work in 1900. The units of heredity of Weismann had not been tested out because they were not conceived as being correlated with visible characters. The fact that characters could be sorted out after they had entered into combination with other characters is unquestionably the strongest appeal in Mendelian interpretation. A temporary eclipse of a unit character does not rob it of its individuality. The units of heredity are carried in the germ cells. The germ cells alone are concerned in the transmission of hereditary qualities. In the Mendelian analysis of the nature of inheritance we have Weismann's germ plasm and determinants. Weismann predicted a reductional division, and the animal and plant cytologists found his prophesy to be true. Units of heredity are located in the germ plasm—in the chromosomes of the germ plasm. By means of a large array of facts gained from breeding, together with the findings of the cytologist, the geneticist has mapped out the chromosomes, assigning to them hereditary units in definite position and arrangement. Chromosome maps, segmental interchange, disjunction, non-disjunction, crossing-over, chiasmata, and genes—these are some of the words of the new preformationist doctrine.

There was no room in Weismann's theory for the inheritance of somatic modification save when this modification left an imprint on the germ plasm. New characteristics could be perpetuated only if the germ plasm dictated it. The workers with *Drosophila*, *Datura*, and *Nicotiana* achieve morphological modifications through the shifting about of the chromosomes, through the piecing together of parts of chromosomes, through mutations (natural or induced), and through permanent re-alignment of genes. In neither Weismannism nor Mendelism is there a mechanism which would allow for the inheritance of acquired characters. Recently there have been two additions to the picture: Belling (1931) identified the chromosomes with genes, and Gowen (1933) has succeeded in measuring the size of the gene.

The picture is now complete. The chromosomes of the germ nuclei are permanent structures. Their number is constant. The genes are located in the chromosomes. They are arranged in definite order. They are definitely spaced. To look at a chromosome map of *Drosophila* or *Datura* is to see the preformed complex embryo in the egg and in the sperm.

All preformationist theories of development must assume qualitative and quantitative differentiation based on chromosomal reduction in one form or another. And that can occur only when a cell that is to be differentiated receives something less than a non-differentiated cell. A differentiated cell can no longer be a totipotent cell in the sense that it could, if the occasion should arise, reproduce the individual of which it is a part. Boveri's demonstration of a somatic reduction in the cleavage stage in *Ascaris*, where body cells are differentiated from germ cells, by an obvious rejection of chromatic material, gives cytological proof for such an assumption.

In the very field of embryology which apparently lends support to preformationism, data have accumulated which strike at the foundations of that concept. Using experimental methods, a large array of investigators have found that differentiation does not rob the cells of their potentialities, that parts of what was an organized whole can create a complete individual anew. I quote from Dürken (1932, p. 208-209), who has admirably summarized the facts in his "Experimental Analysis of Development":

"In considering the determinative interrelation within the germ (zygote before differentiation), it must not be forgotten that determination as a whole is a function of the whole germ, and not simply the sum of the action of its morphological subdivisions. Attention has already been drawn to the fact that the fate of a part in development is always decided 'within the framework of the whole' and with reference to the whole. Not only is there a separate activity of the parts, but there is also an action of the whole, since development is epigenetic—i.e., is not merely the unfolding of a diversity that is already present in its entirety, but the production of a manifoldness that is really new. To say that determination as a whole is the sum of separate interaction would be to abandon the result of the analysis of development, and return to a purely preformationistic conception of development. In view of what has been advanced in the earlier part of this book, that is not possible."

Regeneration, transplantation, implantation, and explantation experiments have been found to yield valuable morphogenetic clews. In induced regeneration the new structure is formed from a blastema which originates from indifferent cells in the immediate vicinity of the wound. Self-induced regenerations also occur. An organism may throw off single parts or organs and form new ones in their place. The fact that a completed adult individual may, after the loss of an organ, produce another one in its place shows that cell potency has not been lost during or after differentiation. The removal of an organ may not necessarily result in the formation of a similar organ. In the saw-fly *Cimbex axillaris* a regenerated antenna may bear a claw at the end. In the decapod *Palinurus vulgaris* an amputated eye may be replaced by an antennula.

In transplantations parts of the body are removed from their normal position and therefore removed from the coordinating influence of the organism as a whole. The behavior of the graft elements in the new environment can be watched. Pieces of what would normally become epidermal tissue develop into brain and muscle tissue, if removed from a young amphibian embryo and substituted for tissue in other regions (Spemann, 1929). A bit of transplanted blastopore lip may result in secondary embryonic regions with brain, dorsal groove, eyes, ear pits, chorda, and larval skeleton (Spemann, 1929). A piece of older epidermis of a frog embryo (anterior abdominal region) placed in the future mouth region of an equally young salamander embryo may result in the formation by the salamander embryo of mouth organs of the frog type (Spemann, 1929).

The embryonic optic cup is essential for the formation of the lens of the eye. The optic cup itself is replaceable in the early embryonic stages by foreign material; this indicates that it is dependent upon some sort of organizer. The lens originates from the outer epidermis in the vicinity of the optic cup. When the presumptive rudiment of the optic cup in an Anuran embryo is removed before the development of the lens, the lens does not, as a rule, develop. If the region of the epidermis where the lens is formed is replaced by a piece of epidermis from the trunk of *Rana esculenta*, a lens is formed. When the primary optic cup is transplanted to another region, a lens will be produced from the epidermis in the vicinity of the transposed optic cup.

The random examples cited here, taken from observations in experimental embryology, show how plastic the animal body is, even after differentiation has set in. The partners of a graft may change the direction of differentiation. The fact that the epidermis of one animal grafted onto that of a non-related animal may influence morphogenetic expression in the host shows that even a part of an organism contains organizing powers. It shows also that the organizer of a given part can pass beyond the barrier of cell boundaries. #

Such organizing substances we find elaborated in glands. The rôle of hormones is too well known to bear repetition here. The outstanding fact about the hormones is that they, as chemical messengers, remote from their seat of origin, influence morphological expression in a very marked degree. Metaplasias in animal forms may occur at any time in the cycle of the organism. Sarcomas in the animal body tend to appear after maturity has been reached.

The unitary concept of development in the light of accumulated contradictory data breaks down, and no matter how many props it may receive, it still is a part of a preformationist architecture whose plans can never be made sufficiently elastic to meet the emergencies and needs of an organism whose plans change from moment to moment as it increases in complexity.

The evidence from the plant kingdom, because of greater plasticity, is even more convincing than that in the animal kingdom. The botanical literature of the last fifty years is very rich in material dealing with experimental morphology. Horticultural and agricultural practices reach so far back that we shall never know their origins; yet vegetative propagations of diverse kinds have been handed down as cultural heritages in all parts of the world where plant domestication has taken place. Actual experimentation in morphogenesis is recorded by Malpighi. Knight, Kny, Van Tieghem, Sachs, Klebs, Voechting, Raciborski, Blaringham, Figdor, and McCullum are some of the botanists who have contributed to our knowledge in that field.

From the large list of modifications that have been brought about experimentally, the following facts have been secured:

Modification of leaf form and size may result from controlled environment, such as light, temperature, moisture, etc.

Modification of internal structure of the leaf can be brought about by controlling the environment, such as light.

Modification of growth habit of the plant as a whole may result from controlled environment, such as light, temperature, moisture, etc.

Modification of sex expression may be brought about by controlled environment, such as light, temperature, moisture, etc.

Modification of internal structures of root and stem may be brought about by controlled environment, such as light, temperature, moisture, etc.

Modification of growth habit may be induced through removal of the main branch or the main root.

Injury may lead to the formation of adventitious buds on stem, roots, and leaves.

Modifications due to regeneration may result in the formation of substitute organs: (a) prothallus from injured moss capsule, (b) roots from callus at base of leaf or stem, (c) plants from injured leaves, (d) sex reversals.

Modification due to grafting may result in sectorial and true chimeras.

Differentiation in plants does not necessarily deprive the cells of their totipotency. Whatever the causes may be that shape the form of a leaf, the position of a root, the appearance of a flower, the polarity of a plant, or the kind of cells that are present in organs—the decrees of those forces may be ruthlessly upset and reversed. And because the number of instances are so wide-spread, genic control of morphological and physiological expression of an organism seems like a naïve explanation. That parts of the plant are conscious of the organic whole is apparent from the integration of parts resulting in a balanced organism. How long that balance may be maintained cannot be said with any degree of certainty. The fact that a balanced entity like the rose bush with its sharply delimited organs—roots, stems, leaves, and flowers—can be cut into a very large number of parts and from each part an organismal whole can be reclaimed, shows that chromosomal dictation has been overemphasized. In plants more than in animals, a state of equilibrium can be toppled over and a rearrangement brought about to form another state of balance. Segmenting animal eggs, whose separated blastomeres may each give rise to a fully formed embryo, show a limitation in their ability to form the individual anew when separation is attempted after a certain number of divisions. This is not true in the plant kingdom. The higher plants, in contrast to higher animals, never reach great complexity of differentiation. Such a condition is decidedly in favor of an evaluation of morphogenetic forces. It will be shown later that Harper's (1918, 1928, 1929) morphogenetic studies with lower plant forms have gone far beyond the observations of the experimental morphologists who have preceded him. He was able to watch development go on before his eyes, and he was able to observe organisms march toward complexity, and when the climax was reached, complexity was resolved into simplicity—a preparation to begin the cycle all over again.

Graft-hybrids in plants present a morphogenetic problem whose significance parallels the grafts in animal forms but whose historical significance has not been paralleled. We know of no graft hybrids in animals where the span of life equals the historic *Cytisus Adami*, presumed to be more than one hundred years old. As we know now, the glove-finger relationship between the tissues involved gives to the resultant an intermediate character. The hereditary potentialities of both elements maintain themselves in spite of their position, whether on the inside or the outside of the graft. If an opportunity presents itself, the totipotency of the cells becomes evident. The graft-hybrids of *Solanum* and *Lycopersicum* originated by Winkler, more than any other graft-hybrids, have proved to be important analytical material. Cell complexes in contact with other cell complexes of a different genetic constitution produce a functional, physiological, and morphological entity. When the outer layer is nightshade and the inner layers are tomato, an individual is obtained that expresses both parents, but not equally, since organs and tissues arising from epidermal cells will take on the character of the outer layer, whereas organs and tissues arising from hypodermal cells will be more modified since they will have the additional influence of the epidermal layer. When the outer layer is tomato and the inner layers are nightshade, the epidermal organs will be tomato, and the inner organs will be of nightshade tissue, influenced or modified by tomato tissue. When the two outer layers are nightshade and the inner layers are tomato, a more pronounced modification occurs. When the order is reversed, a new set of modifications is brought about. And when layers alternate—such as the first nightshade, the second tomato, the third nightshade, etc.—still another kind of adjustment takes place.

The adjustment is not between two sets of hereditary units, but between two sets of tissues of different hereditary potentialities. The cells of each do not give up their inheritance; the position of the interior cells, whether they be nightshade or tomato, determines their physiology. It is hard to believe that these cells have lost, qualitatively or quantitatively, chromatic material which would make them different. Position apparently determines function and form. The very fact that in graft-hybrids an adjustment is made between cells argues strongly that such is the case in organisms whose cells all have the same origin.

The great proponent of the chemical nature of formative forces was Sachs. His concept of "Formbildendestoffe" included a number of chemical products presumed to influence morphological expression; they were considered to be specific in their action. Haberlandt (1924) believes to have demonstrated the presence of a wound hormone that influences cell division resulting in callus formation. From Went's laboratory a number of papers have appeared which seriously reconsider Sach's point of view. Noteworthy are the contributions of F. W. Went (1928) and Dolk (1930) on growth and growth hormones. The growth hormone which can be secured from

the coleoptile of *Avena* may induce growth in other Gramineae. The hormone passes out of the cut surface of the coleoptile and may be absorbed by gelatine or agar, which then can be cut into small cubes. The cubes containing the hormone applied to seedlings whose coleoptiles have been decapitated bring about growth reaction. A general discussion of this matter is given by F. A. F. C. Went (1932). Laibach (1932), following Fitting's methods, extracted by means of hot water, growth hormones from pollen of orchids and *Hibiscus*. He was able to induce through their agency the curvature of oat coleoptiles and the swelling of the gynoeceum of orchids.

Pathological plant anatomy, ecology, insect galls, and parasitism in general, add to the picture of plant-form lability and strengthen the epigenetic concept of development. I need only to refer to such standard texts on teratology as Masters, Penzig, and Worsdell for further evidence. A very thorough study of water and swamp plants conducted by Glück (1924) over a period of a quarter of a century presents a kaleidoscopic view of form modification due to environment.

The students of cell lineage in animal forms are confronted with the difficulties involved in the rapid differentiation after a leisurely cleavage. As a result, morphogenetic forces cannot readily be separated out. In the plant kingdom the lower forms offer a rich field for experimentation. Harper's (1918, 1928, 1929) observations on *Pediastrum*, *Dictyostelium*, and *Polysphondylium* have shown in a striking manner that morphogenetic factors may readily be sorted out and analyzed.

The life history of *Polysphondylium*, from myxamoeba to spore, can be followed step by step. A population of myxamoebae which move about without any evidence of order or orientation—a population in which each cell is an individualist unaware of neighbors—undergoes a change so that the erstwhile chaos results in order. The individual myxamoebae, as if obeying a rhythmic urge, become mass conscious, and from that moment a coordinated series of events takes place, culminating in a highly organized sporocarp with polar differentiation, metameric and radial symmetry, and apical and lateral sori. In the process of differentiation myxamoebae adjust themselves to achieve a columnar and tapering stipe with branches and sori. In the stipes the cells undergo a metamorphosis; they become vacuolar; they expand to form a parenchyma-like tissue. In the sori they become the oblong dense spores.

These observations of Harper offer a challenge to those who maintain that the cell organization is entirely responsible for organismal expression. The cell aggregates in *Polysphondylium* cannot be considered as representing a cell lineage of the kind that is formed in a segmenting egg. Nevertheless mass interaction results in an organism of considerable complexity. In the final expression of form, environment, through tropistic responses, becomes a molding factor. Even in so simple a form, the change in rhythm that a single myxamoeba shows when it becomes conscious of polarity, indicates

how complex and inexplicable the internal re-alignment of such a cell is. And equally remarkable is the phenomenon of mob psychology which grips the whole population and leads it to a goal. Without a design "before the eye," the cells create a complex structure. And as Harper has shown, a small mass removed from the sporocarp goes through reconstructive processes and ends in a fully formed, but smaller, sporocarp. If one wished to make out a case for an old theory which was never taken seriously, *Polysphondylium* lends support to Semon's (1912) memory (mnemonic) theory, which Hering advanced sixty years ago.

In forms of life where cell numbers are achieved through the successive divisions of a single cell, the morphogenetic problems increase with increase in cell population. The problems that an individual cell has to face are unique for that particular cell. When two cells, each with unique problems, arise after fertilization, the uniqueness of the one impinges upon that of the other so that the peripheries of their activities meet and achieve a compromise. In that way the problem of contiguity with physiological and spacial adjustments is solved. Whatever new problems are to arise are now shared by two cells. The cells, however, have the irresistible urge to divide, and then there are four cells. Again there are adjustments and compromises in which all cells share. Four cells become eight cells. The individualistic cell concept becomes relegated into the background, and the organismal concept begins to loom up. The population increases; the cells are held together. There is the heed of polarity; there are upper cells and lower cells. There is the heed of surface tension and space relations, problems that Matzke (1927) and Hein (1932) have recently emphasized. There are cells on the inside, cells on the outside; there are totally compressed cells and partially compressed cells; all of which results in a division of labor. As the organism grows, it expresses an individuality achieved through the integration of a great number of cells. With the increasing complexity of the organism, more and more cells lose their ability to reproduce the organism in its entirety should the situation arise.

Zeleny (1933), in his effort to explain changing developmental processes in the serpulid worm in terms of the gene, presents evidence which may readily be applied to support the epigenetic concept of development. Using the illustration of the development of gills in four groups of worms represented by *Protula*, *Apomatus*, *Serpula*, and *Hydroides*, he shows that complexity of those organs increases, beginning with *Protula* and ending with *Hydroides*. In their individual life cycles members of the fourth group, *Hydroides*, pass through stages resembling the adult stages of the other three groups. These stages are passed through not during larval development but after the worm has become sedentary and has developed its calcareous tube. Furthermore, the production of the gills of the first three groups before the final hydroid gills are produced involves a radical destruction of the first type before the second type is produced, a radical destruction of the second type

before the third type is produced, and a radical destruction of the third type before the fourth type is produced. "That the structural differentiation at any stage is not merely part of a sequence of necessary events in the erection of adult structures is evidenced by the fact that regeneration of the removed functional organ (whether gill without opercula modification, *Serpula* type or *Hydroides* type of operculum) gives a replica of the removed organ *without recapitulatory stages*." Zeleny found no difficulty in explaining such phenomena by assuming that the same gene may act differently under different physiological stimuli, and that similar genes in different parts of the body may, because they are subjected to different physiological agents, produce strikingly different somatic results. Yet his own data show that once a certain level of organization has been reached (a higher level of organismal complexity) there is no retracing of steps.

In the development of the leaf in the palm *Elaeis guineensis* (Yampolsky, 1922) various epigenetic levels are achieved by the plant. The first seedling leaves are undivided. As more and more leaves are produced, division of the leaf blade (a true lamina) begins. At first two-parted, the later leaves become more and more divided, so that the climax is reached when a leaf four meters long is produced with over 160 pairs of leaflets. Even in the undivided juvenile leaves, leaflet "Anlagen" are present but are held together by the original tissue of the lamina. The increase in the number of leaflets is brought about by the production of an increasing number of leaflet primordia. It takes five years before an oil palm tree reaches the highest level in leaf development. Whatever the forces may be that are responsible for the end results, a leaf four meters long could not have been achieved without the parallel development of other parts of the plant, stem, and roots. A correlative epigenetic development of all parts of the plant results in an integrated adult organism. Hence, when that point is reached, all the leaves produced from then on will be more or less alike.

Again I have taken random examples, this time from the plant kingdom, to show how plastic the plant body is, and how readily it is modified. Grafting, regeneration, control of environment, mutilations, hormonal influences, parasitism, and metaplasias are molds which may profoundly alter the plant body.

For many years I have had the plant *Mercurialis annua* under observation, and I have seen it express itself in many forms. I have witnessed plants and parts of plants molded into curious patterns. I have taken some of these patterns apart, hoping to discover therein the answers to the questions that I have propounded to myself.

Sex intergradation in *Mercurialis annua* exhibits itself in every conceivable fashion. Whole plants show gradations between the extremes of male and female; flowers and other parts of plants show intergradations. The axillary female flowers are, as a rule, two- and often three-carpelled. These are, in the main, sessile structures. I have described female flowers on

elongated peduncles (Yampolsky, 1930). The male flowers on the male plants are borne on interrupted spikes which surpass the leaves in height. Male flowers may also appear as sessile structures borne in the axils of the leaves of female intergrades. The female flowers are apetalous, green, two- or three-carpelled—each carpel with a single ovule. The style may be two- or three-parted with white, translucent, blunt stigmatic hairs. There are two nectaries present. The male flowers are apetalous with from eight to twenty stamens. Each stamen consists of a two-sacked anther and a slender filament. There is no evidence of an aborted ovary.

Intergradation between male and female flowers exists in many combinations. Such flowers we may call hermaphrodites. I have observed two-carpelled flowers with one stamen, with two stamens, and with three or more stamens. I have observed flowers with one carpel; the other carpel was replaced by stamens. I have also seen flowers one half of which were composed of carpel and stamens, while the other half consisted of stamens only. I have also seen three-carpelled flowers in various combinations with stamens. The sex intergradation exhibited by the whole plant or part of the plant exists even in the more intimate parts of the flower. Such intergradations involve a reshifting of sex elements in a more or less sectorial arrangement.

Sex intergradation may overstep the bounds of differentiation and result in the simultaneous appearance of conflicting sex elements in an already sex-differentiated tissue. Pistillody and staminody in *Mercurialis annua* are a common occurrence. The following are some of the transitional stages found:

- (1) Anther sac growing out of ovary wall; ovule normal; pollen grains plump.
- (2) Anther sac within the ovary; ovule present; pollen grains plump.
- (3) Three-carpelled ovary normal; three stamens with two anther sacs each; upper part of anther sac with stigma, style, and characteristic trichomes of female flower.
- (4) Two-carpelled ovary with stamens transformed into completely sterile tissue, but with stigma and style.
- (5) Two-carpelled ovary completely sterile; stigma and style present; anther sacs with healthy pollen growing from ovary walls.
- (6) Three-carpelled flower; parts separated; no ovules; anther sacs imbedded in ovary wall.
- (7) Normal ovaries; ovules present; style elongated bearing two anther sacs with healthy pollen.
- (8) Two-carpelled ovary; one fertile, one sterile; one normal stamen.
- (9) Two-carpelled ovary; one carpel sterile; other carpel transformed into stamen with exaggerated filament.
- (10) Two-carpelled ovary; ovary with anther sac; ovule normal; two stamens with stigmatic surfaces.
- (11) Two-carpelled ovary with two stamens with exaggerated filaments.
- (12) Single-carpelled flowers; stamen with filament and anther sacs growing out of ovary wall.
- (13) Three-carpelled flower; stigma and style present; carpels separated; ovules aborted; two anther sacs from each ovary.
- (14) Male flower with twelve stamens; one with stigmatic surface.
- (15) Male flower with more than one stamen with stigmatic hairs.

- (16) Male flower; one stamen transformed into carpel-like structure with stigma and style but no ovule.
- (17) Male flower with more than one stamen transformed into carpel-like structure, with stigma, style but no ovule.
- (18) Male flower with all stamens normal except one, bifurcated, one part with two anther sacs, the other part with ovary, ovule, and anther sacs.
- (19) Male flowers with two stamens transformed into sterile carpels; one anther sac growing out of each ovary wall.
- (20) Male flower with all stamens showing various degrees of transformations into carpel-like structures—stigma, style, and trichomes present.

The list is a very fragmentary one, since no attempt has been made to indicate the many other gradations that exist. It is, however, sufficiently representative to bring out the fluctuating character of sex expression in flowers. In an effort to make a cytological study of intergrading flowers, a large number of buds were taken from plants that showed a tendency toward pistillody and staminody. These were fixed in Flemming's medium solution and stained with Flemming's triple stain. As a result, a cytological study

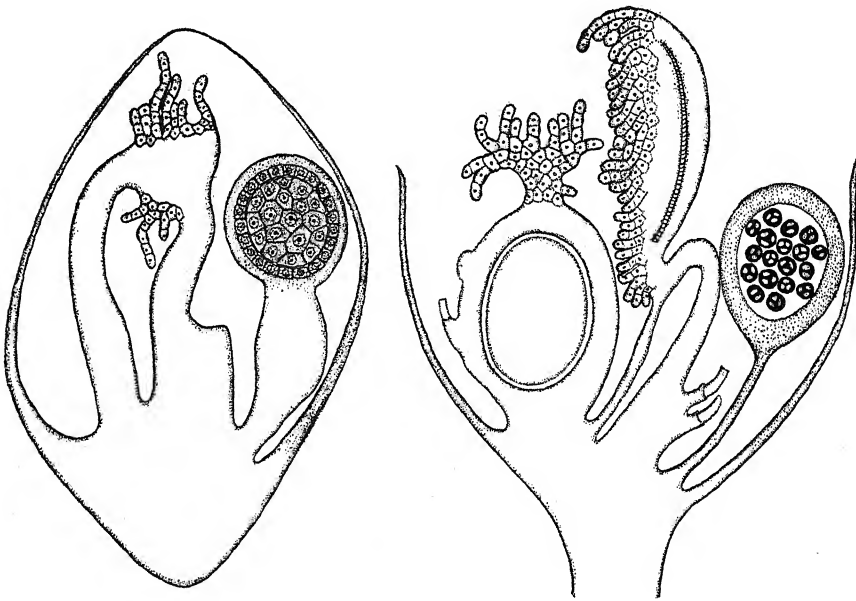


Fig. 1 (left). Female flower—one carpel sterile, the other changed into stamen-like structure. Fig. 2 (right). Hermaphroditic flower with two carpels and one stamen. One carpel with overdeveloped stigma and style, ovule absent.

of intersexual flowers was made possible. A surprisingly large number of buds showed transitional stages, so that our fragmentary knowledge of the cytology of sex intergrades can now be extended.

In describing the various flower intergrades, I shall follow no particular order, since I have selected only a few cases. Figure 1 represents diagram-

matically a female flower one carpel of which is sterile; the other carpel has been metamorphosed into a stamen-like structure. The two parts of the carpel are separated. The sterile carpel possesses a well developed ovary with complete tissue differentiation. No ovule is present, nor is there an indication of an aborted one. I have examined intersexual female flowers with aborted ovules all of whose cells were dead, inside of the living and growing ovary. The carpellate character of the structure in figure 1 is unmistakable, first because of its form and second because of the characteristic stigmatic cells. Inside the ovary there are also stigmatic cells which may owe their origin to the specialized cells of the "obturator"—structures found inside the Euphorbiaceous carpels that are presumed to direct pollen tubes toward the micropyle of the ovule.

How far back carpel differentiation took place we have been unable to trace. We know, however, that the gynoeceium initial possessed something which led it toward the fashioning of sexually conditioned cells. It is evident that the urge toward sexual expression appeared long before the production of the female gamete. It would be easy at this point to speak of a female sex organizer that influences the formation of ovary walls, stigma, style, obturator, ovule, integuments, and embryo sac. But as I shall show later, the male flower which does not possess even a rudiment of a gynoeceium may be changed entirely or in part into a female flower. Where did the female organizer come from that changed maleness into femaleness? The students of sex in plants and animals very often fail to see that the change in rhythm in an organism which culminates in the production of a gamete is most likely the essence of sex, and bringing gamete and gamete together is of secondary consideration. This sterile carpel did not complete its sex cycle; something intervened and deflected the course of its expression. The misshapen ovary and the stigmatic hairs bear witness to that fact.

I have already stated that the gynoeceium initial became sexually conditioned and then moved toward the expression of femaleness. From that cell other cells have arisen that express femaleness, and then there is a sex reversal. One of the carpels becomes a stamen with anther sac containing pollen mother-cells and a filament-like structure which is really a solid mass of ovarian tissue.

The forces that caused the gynoeceium initial to become different from the other cells and sent it toward a specialized kind of expression could not make it forswear its dual nature; the residual maleness asserted itself. We thus see a carpel, the lower part of which retains the carpellate character and the upper part of which is transmuted into an anther sac with anther sac morphology and physiology. In no way can the broad cylindrical structure be homologized with a filament. The pollen mother-cells in the anther sac were normal. The tapetal cells, with characteristic spherical and oval-shaped prochromosomes, are the same kind that are found surrounding pollen mother-cells in normal male flowers. The prochromosomes in the pollen mother-cells

in this stage are more or less rod-shaped, straight or bent. In a later stage these pre-synaptic figures will be found clumped together with the nucleolus. In the intersexual flower represented by figure 1, potentialities are realized which otherwise would not have had an opportunity to express themselves. Long before reduction division, the gynoecium was differentiated. Was it a sex chromosome that was responsible for female sex expression? If so, why was maleness also expressed?

Figure 2 represents an intersexual flower of another type. There has been no sex alteration here. It is really a hermaphrodite flower with two carpels and one stamen—a type of flower that I have described before (Yampolsky, 1919). Something has happened to one carpel that caused it to make an exaggerated vegetative growth resulting in a greatly overdeveloped stigma and style and a poorly developed ovary with ovule absent. The other carpel developed normally and contained a fully formed embryo sac, an obturator, a stigma, and a style. Both carpels have the hairs or trichomes characteristic of the female flower. The conspicuous feature of this flower is the hyperplasia of the sterile carpel. The stigmatic surface has increased in size, and the style is abundantly supplied with vascular tissue. It is interesting to note that the formative forces affecting the sterile carpel had apparently lost control, finally producing a futile structure. In my polygamous cultures a large number of flowers show intersexual states. I have collected viable seeds from such plants. Many seeds were developed from intersexual flowers in which embryo sac and pollen grains were functional. Petalody of the sex elements of a flower—a form of metaplasia—may not be the result of atavistic ancestral traits that indicate evolutionary origins; but they may indicate potencies realized through organizing influences in the same way that a substitute organ replaces one that has been removed.

Figure 3 represents a female flower with aborted or non-developed ovules. Such a flower is completely sterile. Ovaries and stigmatic hairs are the only indications of the sex of the flower. The independence of the other parts of a flower in development is brought out by the fact that there is no suppression of stigmatic surface, even though the ovule has failed to develop. The floral envelope, the ovary, the style, the stigma, the integuments, and the embryo sac represent distinct waves of development, which may or may not go on simultaneously. The floral envelope may develop even though ovary and ovule abort. I have observed naked ovules barely enclosed by an arrested floral envelope. In one of the sterile carpels of figure 3 the lining of the ovary has been metamorphosed into stigmatic cells, showing again that differentiation may be upset and replaced by another kind of differentiation.

Figure 4 represents a flower that undoubtedly started out to produce a normal gynoecium. Later, there developed two accessory carpels that were transmuted into stamens before ovarian differentiation had taken place. I base my conclusions upon the size and diameter of the filament-like structure in figure 1, which is a modified ovary. The only other interpretation that

we may advance is that the stamens developed from the two nectaries which are always present in the female and hermaphrodite flowers. This two-carpelled gynoecium, except for slight modifications of the stigma and style, was normal and bore on the inside ovules with embryo sacs. The modified

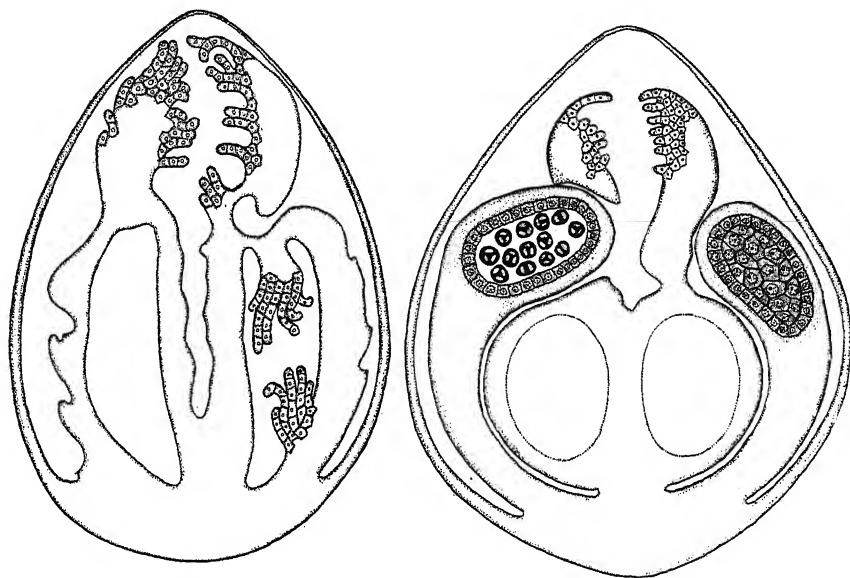


Fig. 3 (left). Female flower—no ovules; one ovule replaced by stigmatic hairs. Fig. 4 (right). Flower with accessory carpels transmuted into stamens.

stamen-like structures to the left and right of the carpels, if they did start to develop simultaneously, failed to keep up an equal pace toward maturity. The stamen-ovary structure to the left is in the uninucleated spore stage with the cells still imbedded in the thickened mother-cell wall. The cells in the anther sac of the stamen-ovary structure to the right are about to enter the so-called synaptic phase of maturation.

In this flower we again see disturbances in equilibrium. They do not, however, hinder physiological manifestations. Viable pollen grains are produced, and functional embryo sacs can give rise to seeds containing embryos. The non-fixity of a given differentiation is evident.

In figure 5 a hermaphrodite flower shows one differentiation followed by a second differentiation. Here staminody and pistillody exist in the same flower. The stamens whose normal filaments are not indicated here show anther sacs with stigmatic cells. The presence of the stigmatic surfaces has not interfered with maturation processes, and the spores in the tetrads are seen enclosed in the mother-cell walls. There are two possible interpretations for this phenomenon. Assuming that there is an organizer—a "Formbildenderstoff"—that traverses through cells to the region where it causes

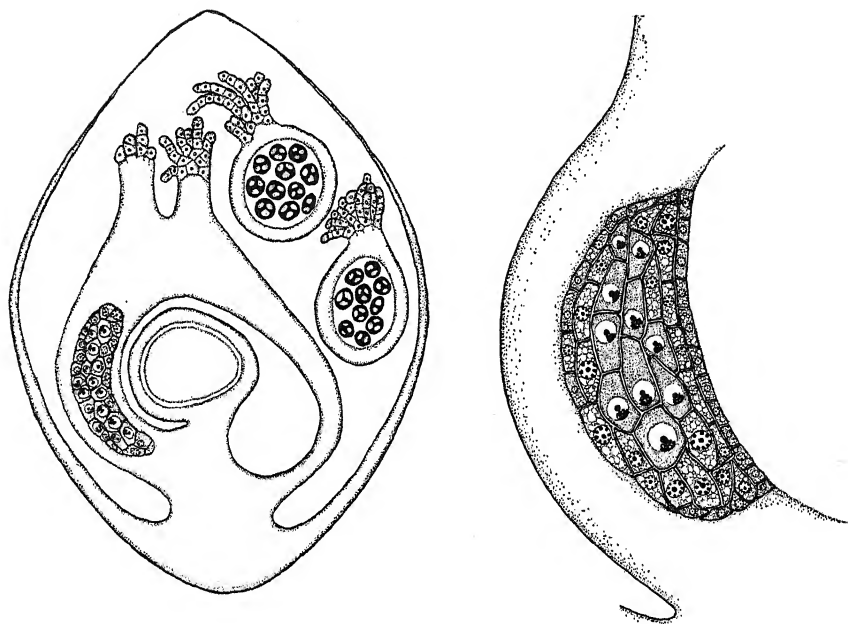


Fig. 5 (left). Staminody and pistillody in a single flower. Fig. 6 (right). Pollen-mother-cell tissue within ovarian tissue.

morphological expression, then that substance must have become deflected from the gynoeceum and have traveled up the stamen and caused stigmatic cells to grow in a region whose pole corresponds with the pole of the carpels. The other interpretation is that the specialized cells of the anther sac wall, like the differentiated cells of the ovary wall, contained latent potencies which were stimulated to express themselves. I have observed stamens whose apices were elongated to form a neck-like style with stigmatic cells at the free end. I have seen stamens in hermaphrodite flowers with trichomes growing out of the filaments. I have also examined stamens in hermaphrodite flowers that have been metamorphosed into a cylindrical structure with stigma, style, and trichomes, but with no trace of anther sac or ovules—sterile female structures. Externally the carpels of the flower shown in figure 5 are to all intents and purposes normal. The section was cut through one ovary and ovule and through part of the ovarian chamber of the second. Judged by serial sections, both ovules were normal in every respect. However, in the ovary wall of one carpel, shown by the crescentic area, unmistakable pollen mother tissue is present. The pollen mother-cells are seen here in the synaptic knot stage with the whole of the chromatic material thrown to one side of the nuclear membrane. Interspersed are tapetal cells recognized by the specific organization of their prochromosomes.

I have dissected many such structures and have examined them under a

binocular microscope. I have found fully differentiated anther sacs with normal pollen grains within the ovary walls. Such anther sacs can be recognized when they are ripe because the yellow shows through the green of the ovary wall. I have also seen anther sacs that protrude directly out of the ovary wall. Maturation of pollen mother-cells and maturation of egg mother-cells may be accomplished in the gynoeceium.

In this very complex flower, parallel and conflicting developmental tendencies act independently. Female tendencies express themselves in the stamens; male tendencies express themselves in the carpels, and yet the original goal is reached. The anther sac contains viable pollen, the ovule contains a functional embryo sac. The clashing developmental tendencies that result in frustration, as is the case in many hybrids widely investigated by Tischler (1908), are seen also in some of the flowers of *Mercurialis annua* with aborted pollen grains or aborted embryo sacs. In cytological evidence of this kind we get a picture of disturbances long before they have reached the gamete formation stage. These disturbances may lead to internal friction but not necessarily to the elimination of one or both of the clashing elements.

In higher animal forms the reproductive organs are complicated structures so that sperms, in order to reach the outside, must pass through long tubules, and the egg, if insemination is to occur outside, must pass through the oviduct with its glands. If the egg is not discharged from the mother body, the sperms must make their way to the egg, and this usually occurs through the agency of a copulatory organ. The reported cases of ovary-testes in animals show the dual nature of sex glands. That one sex may be changed morphologically, physiologically, and functionally into that of the other, we know. For an animal to achieve all the morphological and functional attributes of the opposite sex involves a breaking down of its sex glands and organs and the development of the opposite sex gland and the organs that go with that gland. That necessitates important changes—not merely reversals, but destruction of old parts and the building up of new parts. If that is accomplished, then function and form go hand in hand. Sex glands or sex elements of the opposite sex may be produced in an animal without the accompanying organs that would enable proper functioning. In such cases internal sex-reversal occurs; the products of the new gland circulate in the blood stream and may induce physiological and psychological disturbances in the animal. Intersexuality is present but does not achieve its complement in morphological structures.

Intersexuality in plant forms may and does express itself with ease, because there are few barriers between pollen grains and embryo sacs, and for that reason the tearing down of old organs and the building up of new organs need not take place. In figure 5 the pollen matures inside of the ovary, and the ovule enclosed by that ovary produces a functional embryo sac. Although I have not determined whether a pollen grain produced in an ovary will germinate and send its generative nucleus into the embryo sac, I see no reason why this should not occur.

Figure 6 represents pollen mother-cell tissue within the ovary wall. Cellular differentiation is apparent. The larger cells, with granular, uniform cytoplasm and with nuclear contents obviously in the synaptic knot stage, are the pollen mother-cells. Those with alveolar cytoplasm and nuclei whose prochromosomes are arranged on the periphery of the nuclear membrane are tapetal cells. The single rows lining the inner layer of the ovary are epidermal cells.

Pollen mother cells and tapetal cells must have become secondarily differentiated within an already differentiated tissue. The cells of the ovary represent female tendencies. The expression of femaleness did not, however, as I have previously stated, deprive the cells of their ability to revert to another kind of sex expression. Here we see a cycle within a cycle, carrying on activities which appear more or less independent. The stamen "Anlagen" in the normal flower develop into structures with differentiation such as the anther and filament. The anther with epidermis, parietal layers, tapetal, cells, and pollen grain mother-cells shows further differentiation. During the process of differentiation the pollen mother-cells come to lie on the inside surrounded by the tapetal layer, which in turn is surrounded by the parietal layers, and finally by the epidermis which becomes highly specialized to allow for dehiscence. Fully matured and differentiated anther sacs, as we have seen, are formed inside the ovary wall. Anther sacs inside of ovaries are found where a balanced adjustment is not secured and pollen mother-cells and tapetal cells form a mosaic structure. The cells in the anther sac shown in figure 6 have not solved the problem of balance, so that pollen mother-cells, tapetal cells, and parietal cells are intermingled. In spite of these difficulties, fully matured pollen grains do arise in such imperfectly balanced structures.

Pollen mother-cells may also develop inside of ovules. In such instances the embryo sac mother-cells either do not develop or become replaced by cells that are to become pollen grains. I have no complete picture of the fate of such cells, since I have had but a few stages under my observation. There is no reason why such pollen mother-cells should not form viable pollen grains within the integuments.

Figure 7 represents a female flower with one fairly normal carpel and another carpel in which sterilization, followed by a secondary tissue differentiation, has taken place. The anther sac shows pollen mother-cells with the prochromosomes arranged around the periphery of the nuclear membrane. Tapetal, parietal, and epidermal cells make an almost complete balance of parts. This bi-sexual carpel shows an unusually large stigmatic surface. The fertile carpel was normal save for the overwhelmingly large stigmatic area, which is shown in part separated from the ovary.

Figure 8 represents a male flower some of whose parts exhibit sex reversal. One stamen has been metamorphosed into a carpel with stigma, style, and ovary. One stamen bears at its apex a crown of stigmatic cells; the third stamen is normal. The male flower initial, because it was destined to produce

a male flower, had had its fate decided before it proceeded to express male tendencies. The elements of the flower were differentiated and developed in accordance with a male flower design—an indication of progressive differentiation. Yet sex reversal took place, without the presence of a vestige of female tissue in the flower, which, if it were present, might (if we admit such a thing as an organizer) have stimulated latent female qualities. In the male

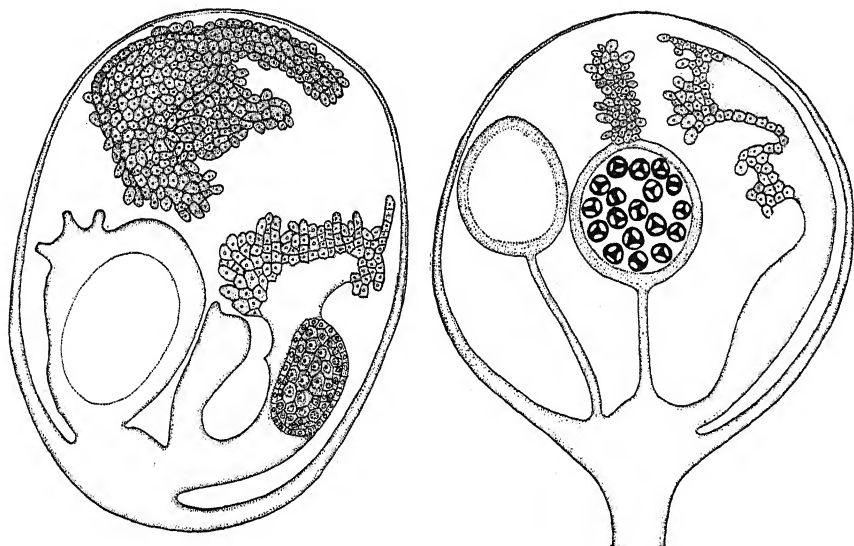


Fig. 7 (left). Female flower—one normal carpel, the other transmutated into stamen with large stigmatic surface. Fig. 8 (right). Male flower showing sex reversal.

flower, just as in the female and hermaphrodite flowers, the greatest range of variability in transmutation of parts occurs. I have found fully formed ovules in stamens of male flowers existing side by side with anther sacs that appeared to be normal. I have also observed stamens that have been changed into pistils with fully formed stamens (anther sacs and filaments) growing out at right angles from the secondarily formed ovary wall.

Sex intergradation is firmly established in *Mercurialis annua*. It is found in the complete plant; it is also found in the smaller units of the plant. How far that intergradation may extend is hard to define. The cytological evidence indicates that it is present in germinal tissue. The genetical evidence indicates that it may extend even into the gametes themselves. It is apparent from all the data that a formula for sex inheritance involving digamety is out of the question. Since alternative sex inheritance receives no corroboration in this plant and because graded potencies in sex lead us into the very gametes themselves, we must conclude that both male and female gametes are as variable in their sex potencies as are the plants to which they give rise.

Preformationism receives no support from *Mercurialis annua*. Modern

preformation, with its genes arranged in chromosomes, pigeon-holed and labelled, can create no blue-print plans to guide in the erection of a plant of this kind. We have seen that *Mercurialis* makes its own plans as it proceeds toward maturity. One cannot deny that there is an inherent formalism in specific protoplasm. Plants and animals cannot escape the pattern of their make-up. What becomes evident, however, from the array of facts is that external forces (nonresident forces) take part and modify the inherent pattern.

If genes and chromosomes do not determine form and function, how can one explain pattern behavior of protoplasm? We have seen that cells in contact adjust themselves on the basis of the least space wasted. Groups of cells in contact tend to take on a fourteen-sided form. Dividing cells tend to have their planes of division in the short axis of the cells. Groups of cells dividing tend to do so in the form of parallel or intersecting parabolas. Differentiated cells may become undifferentiated and behave as embryonic cells, to give rise to new organs or complete individuals and thus reverse the pattern prescribed by the germ cells. Chemical substances, as in the case of animal grafts, may stimulate the production of organs not characteristic of the host. Chemical substances may cause growth curvatures in response to light. Those same substances removed from a stimulated cell and introduced into plants that have not been stimulated can induce a reaction resulting in growth curvatures. Controlled light causes sex reversals. Controlled nutrients result in the suppression or in the acceleration of the production of sex organs. Mutilation results in the production of substitute organs and in sex reversal. Individualistic myxamoebae exhibit mass action and proceed to arrange themselves into a complicated fruiting body.

To look at an organism with stem, branches, leaves, roots, flowers, and fruits—an organism that has arisen from a fertilized egg and has functioned properly—and to say that it is a phenotype (i.e., that its real self lies buried in the germ cells) is to deny the morphogenetic forces that I have just enumerated as molding forces. The preformationist of today is confronted with Wolff's epigenesis. The preformationist believes that he holds his finger to the pulse of life and measures its beat.

I quote from Wilson (1904):

It remains to inquire more critically into the nature of the correlation between growth and cell-division. In the growing tissues the direction of the division planes in the individual cells evidently stands in a definite relation with the axes of growth in the body, as is especially clear in the case of rapidly elongating structures (apical buds, teloblasts and the like), where the division planes are predominantly transverse to the axis of elongation. Which of these is the primary factor, the direction of general growth or the direction of the division planes? This question is a difficult one to answer, for the two phenomena are often too closely related to be disentangled. As far as the plants are concerned, however, it has been conclusively shown by Hofmeister, De Bary, and Sachs that the growth of the mass is the primary factor; for the characteristic mode of growth is often shown by the growing mass before it splits into cells, and the form of cell division adapts itself to that of the mass: "Die Pflanze bildet Zellen, nicht die Zelle bildet Pflanzen" (De Bary).

SUMMARY

Differentiation does not necessarily deprive the cell of its totipotency. Whatever the causes may be that are responsible for leaf form, position of root, polarity, the character of the cells of an organ, and the like, the decrees of those forces may be ruthlessly upset and reversed. The ease with which balance is upset in the plant *Mercurialis annua* makes it suitable for morphogenetic studies. The work concerns itself with an intimate examination of the flowers where form and function may be overthrown and reversed.

Carpels are changed into stamens; stamens are changed into carpels. Differentiation is followed by a second and equally important differentiation.

Accessory carpels, in themselves an evidence of change of organization, may be transmuted into fertile stamens.

Highly differentiated ovarian tissue apparently does not lose its totipotency but proceeds to form pollen-mother tissue and ultimately viable pollen grains. Maturation of pollen grains and embryo-sac takes place in the gynoecium. Likewise maturation of embryo-sac and pollen grains may occur simultaneously in the androecium.

Cytologic evidence shows that sex intergradation is present in the smaller units of the plant. The genetic evidence indicates that it is present in the gametes themselves.

Modern preformationism receives no support in *Mercurialis annua*. That inherent formalism is present in a specific protoplasm cannot be denied. What becomes evident from the array of facts is that non-resident forces modify the formalism of protoplasm.

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A THREE-WIRE THERMOCOUPLE SYSTEM FOR USE IN CRYOSCOPIC INVESTIGATIONS¹

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The purpose of this paper is to describe a new type of thermocouple system specifically designed for use in cryoscopic investigations. The system as described has been found by the writer to be unusually well adapted for cryoscopic work. It is believed that a general use of this type of thermocouple system, or other similar set-ups, in studying freezing point depressions of living tissues will tend to displace the better known methods wherein expressed plant saps are used to study the freezing point depression values of the saps of plant cells or tissues.

The majority of botanical workers interested in cryoscopic investigations have used expressed saps and some suitable thermometer such as a Beckmann or Heidenhain thermometer. Various methods have been suggested and used for obtaining plant saps. A summary of such methods has been given by Meyer (1929). All such methods are subject to the same general criticism—namely, they deal with the sap outside the living cell and after it has been subjected to treatments which undoubtedly result in serious modifications of its properties. From the data thus obtained inferences have been drawn relative to the osmotic values of the sap in living cells and tissues. When one considers that very few data are as yet available regarding the changes which occur in the sap when tissues are treated and the sap is expressed, the desirability of cryoscopic investigations based on freezing point determinations made *in situ* in living plant tissues becomes apparent.

The freezing point depression of living plant tissues may be determined by placing the bulb of a suitable thermometer in contact with a mass of the plant tissue and inserting the thermometer and tissue into a freezing chamber. This may be accomplished by wrapping the thermometer bulb with the tissue (whole leaves) or by inserting the thermometer bulb into a hole of the proper diameter cut into a massive tissue (potato tuber, etc.). By these two methods Müller-Thurgau (1880, 1886) studied the freezing point depression values of living tissues of several plant species and, on the basis of his investigations, was able to point out differences in the freezing point depression values of living and killed tissues for several species of plants. Some limitations of such methods are that relatively large masses of tissue are required and that intimate contact between the tissue and all parts of the surface of the thermometer bulb cannot be accomplished quickly and easily with any great degree of certainty.

¹ Papers from the Department of Botany, the Ohio State University, No. 327.

A few botanical workers have made use of thermocouple systems in cryoscopic investigations. A thermocouple system may be used as a substitute for the thermometer in freezing point determinations, either in working with expressed saps or with living tissues. The use of the thermocouple in cryoscopic determinations on expressed saps, however, involves the same sources of error as are found in the more common methods where a thermometer is used.

Dixon and Atkins (1910) and Dixon (1911) described a method of using thermo-electric junctions in the study of osmotic values of expressed plant saps. More recent workers employing thermocouple systems in cryoscopic investigations, however, have used plant tissues in preference to expressed plant saps.

Maximov (1914) described a method of determining the freezing point depression values of living and killed plant tissues by means of thermocouples. He used glass-supported copper-constantan thermo-electric junctions and a high-sensitivity galvanometer. In many ways his set-up was very similar to the set-up described in this paper. Maximov (1914) also presents data for several species to show the change in freezing point depression values for plant tissues when killed by freezing. Other and more recent investigators who have used thermocouple systems in cryoscopic investigations are Wright and Harvey (1921), Carrick (1921, 1930), and Fernald (1931).

A critical use of the method of inserting the thermocouple junction directly into the living tissue and determining the freezing point depression of the living tissue should throw some light on the general question of the osmotic value of the cell sap as it is found in living tissues, and may also be expected to contribute to our knowledge of the nature and magnitude of the changes that occur in the sap when it is expressed as is ordinarily done in cryoscopic work. It was for the purpose of further investigating the osmotic values² in situ and the changes that occur in the tissue when that tissue is injured or killed by freezing that the 3-wire thermocouple system described in this paper was designed and constructed.

Principle of the thermocouple. When any two metals or alloys are in contact with each other, an electrical potential is set up at the point of contact. The magnitude of this potential depends largely on the nature of the two metals or alloys and is practically independent of the size of the junction. Such a junction is known as a thermo-electric junction, thermocouple junction, or thermo-element. Two such junctions properly connected constitute a thermocouple system. For any combination of metals or alloys the magnitude of the electrical potential developed in the circuit of the thermocouple system is proportional to the difference in temperature between the two junctions

² It has been assumed in the discussion of the present work that the osmotic value of the sap in a living tissue is related to the freezing point depression of that tissue in the same way that the osmotic value of an aqueous solution is related to the freezing point depression of that solution. Further investigations will be necessary to establish the limitations, if any, to the validity of this assumption.

involved. The current or amperage, however, is more nearly proportional to the size of the junction and is nearly independent of the temperature difference between the two junctions. A thermocouple system is not used to measure temperature at a given point but rather to measure the difference in temperature between two points, the temperature at one point being known. The thermo-electric junction placed in the medium of known temperature, usually at 0°C. , is known as the reference junction, and by the magnitude of the galvanometer deflection or potentiometer reading the difference in temperature of the two media in which the junctions are inserted is determined.

Galvanometer and potentiometer-galvanometer systems. To measure the temperature difference between the two thermo-electric junctions of a thermocouple system, two acceptable methods are in use. The thermocouple lead wires may be connected to a potentiometer and the electrical potential measured directly, a galvanometer being used to determine the zero point; or the lead wires from the thermocouple system may be attached directly to a galvanometer without a potentiometer, in which case the temperature difference between the two junctions is measured by the magnitude of the deflection of the galvanometer when the circuit is completed. Each of these two methods has its advantages and its limitations; thus the method to be used will depend somewhat on the particular problem at hand for which thermocouples are being used.

The potentiometer-galvanometer method, a discussion of which will be found in the paper by Robinson (1927), seems to be the method preferred by the majority of investigators using thermocouples in biological work. For measuring the temperature of a body whose temperature is nearly constant or slowly changing, this method is quite satisfactory and well deserves its popularity. Perhaps the greatest advantage of the potentiometer-galvanometer system is that a very sensitive junction can be used over a wide range of temperatures. Another advantage of this system is that it is relatively less affected by the length and temperature of the lead wires than is the system in which a galvanometer is used alone.³ The chief disadvantage of

³ This difference is due primarily to the difference in the unknown value being measured in the two cases. With the potentiometer system the electrical potential between the two junctions is measured directly. The length and temperature of the lead wires have relatively little effect on the magnitude of this potential, as measured by the potentiometer. On the other hand, the galvanometer as a deflection instrument does not measure the potential directly, as the galvanometer deflection is not strictly proportional to the temperature difference between the two junctions unless the amperage and resistance of the system are maintained at constant values. The deflection of a galvanometer is not proportional to the potential (voltage) alone, nor to the current (amperage) alone, but to some function of these two values. Changes in length and temperature of the lead wires appreciably alter the resistance of the system, and this change in resistance has relatively more effect on amperage values than on voltage values. Thus the values obtained when the galvanometer is used as a deflecting instrument are more affected by changes in length and temperature of the lead wires than are the values obtained in using a potentiometer-galvanometer system.

the potentiometer-galvanometer method is that a rapidly changing temperature can scarcely be followed because of the operator's inability to adjust the potentiometer fast enough to follow the rapidly changing potential. In investigating the freezing point depression values of plant tissues, it has been found in the present work that in using blocks of tissue $3 \times 3 \times 3$ mm. suspended in a freezing air chamber at about -20°C . the temperature change of the tissue on freezing is so rapid as to render the use of a potentiometer-galvanometer system difficult or impossible. In addition to the difficulty of following a rapidly changing potential with the potentiometer, it has been found in using these small blocks of tissue that, after undercooling, the temperature rises to the freezing point very rapidly and remains at its maximum⁴ for only a few seconds. This extremely short period of maximum temperature rise after undercooling makes it quite impossible to determine accurately the point of maximum temperature rise by means of the potentiometer-galvanometer system. This objection, however, would not apply if sufficiently large masses of material were used. With the thermocouple system attached directly to a galvanometer, however, the rapidly changing temperature can easily be followed even when much smaller blocks of tissue are used. The ease with which a rapidly changing temperature can be followed is perhaps the greatest advantage of the galvanometer system. The greatest disadvantage of the galvanometer alone for measuring temperature differences between the two junctions is that, for any given set-up, as the sensitivity of the system is increased the range over which the temperature of the unknown or test junction can be followed is correspondingly decreased.

In using the thermo-electric method of determining the freezing point depression values of plant tissues, it was found that the point of maximum undercooling was seldom lower than -10°C ., and that the uncorrected freezing point depression for most species so far investigated was seldom greater than 3°C . To combine a reasonable sensitivity over a range of at least 10°C ., to follow the undercooling, and a high sensitivity over the range in which the tissues froze after undercooling, the 3-wire junction described in the following pages was constructed. With this system it is possible to follow the undercooling to the nearest 0.01°C . over the temperature range from 0°C . to -11.42°C ., and, by changing to the more sensitive circuit when freezing commences, to determine the uncorrected freezing point depression to the nearest 0.001°C ., if the uncorrected freezing point depression does not exceed 3.125°C . When the uncorrected freezing point depression was found to be greater than 3.125°C ., the less sensitive circuit was used and the uncorrected freezing point depression recorded only to the nearest

⁴ If the point of maximum temperature reached when freezing commences in the undercooled tissue can be shown to be such that the corrected freezing point depression of that tissue is independent of the extent of the undercooling, the point of maximum temperature reached after freezing commences may be regarded as the true uncorrected freezing point of that tissue. Above a certain minimum size of tissue block this has been found to be the case. See also footnote 6, below.

0.01°C., rather than to 0.001°C., as in the case of the more sensitive circuit. Although the tissue may undercool as much as several degrees centigrade per minute and rise through several degrees to the freezing point in only a few seconds when freezing does start, no difficulty has been found in following such a rapidly changing temperature with this system. The speed and accuracy with which the point of maximum undercooling and the point of freezing can be determined are the outstanding advantages of this 3-wire system in investigating the freezing point depression values of plant tissues of many kinds.

Metals or alloys for the junctions. For the two circuits of a 3-wire thermocouple system to differ in sensitivity, it is necessary to have three different metals or alloys united to form one 3-wire junction. This 3-wire junction is then connected to two 2-wire reference junctions as shown in figure 1. In order that the two circuits may be used to measure the temperature at essentially the same point, the 3-wire junction is to be preferred to the

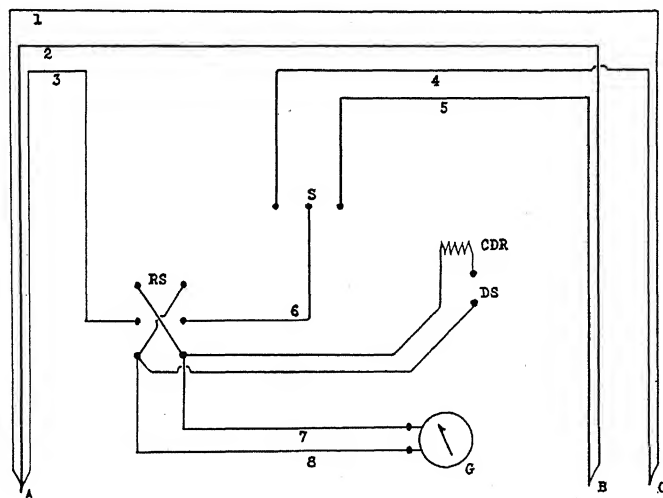


Fig. 1. Wiring diagram for 3-wire thermocouple system. 1, 40-gauge german-silver wire from the 3-wire junction A to the reference junction C. 2, 28-gauge nickel-silver wire from the 3-wire junction A to the reference junction B. 3, copper wire from the 3-wire junction A to one center terminal of a double pole, double throw reversing switch RS. 4, copper wire connecting the reference junction C with one end terminal of a single pole, double throw switch S. 5, copper wire connecting the reference junction B with one end terminal of a single pole, double throw switch S. 6, 14-gauge copper wire connecting the center terminal of the single pole, double throw switch S with one center terminal of the double pole, double throw reversing switch RS. 7 and 8, 14-gauge copper wires connecting the two end terminals of the double pole, double throw reversing switch RS with the two binding post connections on the galvanometer G. S, single pole, double throw switch used to close either of the two possible circuits. RS, a double pole, double throw reversing switch. G, galvanometer. DS, critical damping resistance switch, CDR, critical damping resistance coil (15 ohms).

alternative method of having two independent 2-wire test junctions. The ultimate choice of the wires to be used depends on the sensitivity of the galvanometer and on the temperature range and sensitivity desired in each of the two circuits.

For the two circuits in the 3-wire system now in use by the writer two different german-silver alloys were used against copper. The 28-gauge⁵ german-silver wire used was found to have a thermo-electric potential against copper of about 20 microvolts per degree centigrade. This 28-gauge german-silver wire was obtained from the Kauffman-Lattimer Co., of Columbus, Ohio, under the name of "nickel-silver." The 40-gauge german-silver wire used was found to have a thermo-electric potential against copper of about 5.5 microvolts per degree centigrade. This 40-gauge german-silver wire was obtained from Baker & Co., Newark, New Jersey, under the name of "german-silver." For the purpose of clarity the 28-gauge german-silver wire will be referred to throughout the following pages as nickel-silver, and the 40-gauge german-silver wire will be referred to as german-silver. The difference in potential, against copper, of these two german-silver alloys is not due to the difference in size of the wires but rather to differences in the percentage composition of the two alloys, the thermo-electric potential of one metal or alloy against another being practically independent of the size of the junction. By using these two alloys (german-silver and nickel-silver) against copper, connected as shown in figures 1 and 2, and the galvanometer described below, two different ranges of temperature may be read within the range of the galvanometer deflections.

With the galvanometer described below, the 28-gauge nickel-silver circuit with a 50-cm. scale at $\frac{1}{2}$ meter from the mirror of the galvanometer showed a galvanometer deflection of approximately 16 cm. per degree centigrade. The galvanometer coil is so adjusted that the "zero-point," when the circuit is opened, is at one end of the scale rather than at the middle of the scale. In this way a deflection up to 50 cm. may be observed instead of 25 cm. as would be the case if the "zero-point" were at the usual place in the center of the scale. On this 50-cm. scale with this particular circuit a 1-mm. deflection corresponds to about 0.007°C . difference in temperature between the 3-wire test junction and the reference junction *B* (fig. 1). The telescope used so magnifies the portion of the scale seen through the eyepiece that temperature differences between the two junctions may be read to 0.001°C . with a considerable degree of accuracy. The position of the reversing switch (*RS*, fig. 1) indicates whether the value read is above or below the temperature of the reference junction. In similar manner with the 40-gauge german-silver circuit the galvanometer showed a deflection of approximately 44 mm. per degree centigrade. On the same scale with this particular circuit a 1-mm. deflection corresponds to about 0.018°C . difference in temperature between

⁵ Throughout this paper all references to wire size refer to Brown and Sharpe (B. & S.) gauge.

the 3-wire test junction and the reference junction *C* (fig. 1). With this circuit temperature differences between the two junctions can be read to 0.01°C. with a high degree of accuracy.

The electrical connections of the 3-wire thermocouple system. The 3-wire thermocouple system described in this paper is based on the principle that if two points are connected by three wires, one of which is continuous and the other two selectively interrupted by switches, two and only two possible circuits can be completed between these two points, according to which switch is closed. For a current to flow in either circuit, or for either circuit to show an electrical potential, a source of current or electrical potential must be supplied for each circuit or a common source of current or potential must be supplied for the two circuits. The current or potential developed in the thermo-electric junctions is the only current or potential in this thermocouple system.

The two points *A* and *S* in figure 1 may be regarded as being connected by one continuous and two selectively interrupted wires. The wires 3, 8, 7, and 6 may be regarded as forming a continuous connection between the tip *A* and the center terminal of the switch *S*, through the galvanometer *G* and the reversing switch *RS*. The point *S* (center terminal of the switch *S*) may also be regarded as being connected through the blade of the switch *S* and the thermo-electric junction *B* to the point *A* through wires 5 and 2 when the switch *S* is closed to the right as shown in figure 1. In like manner the point *S* may be regarded as being connected through the blade of the switch *S* and the thermo-electric junction *C* to the point *A* through wires 4 and 1 when the switch *S* is closed to the left as shown in figure 1. The switch *DS* is closed only when the switch *S* is opened to allow the galvanometer mirror to return to the zero point. The use of this switch will be more fully discussed in a later part of this paper.

In this system, as shown in figure 1, the german-silver and nickel-silver wires (1 and 2) from the 3-wire junction *A* are connected directly to the reference junctions *C* and *B*. Two 2-wire reference junctions are used in preference to the alternative method of using one 3-wire reference junction in order to avoid having to insert the circuit selecting switches in the german-silver and nickel-silver lines. It was found to be impractical to insert the circuit selecting switches in the german-silver and nickel-silver lines, as the connections thus formed between copper and these wires acted as thermo-electric junctions and thereby destroyed the usefulness of the system. The possibility of setting up secondary thermo-electric junctions at the switch terminals was eliminated by inserting the circuit selecting switch (*S*) in the copper lines. An additional advantage to be found in having the circuit selecting switch (*S*) in the copper lines is that one single pole, double-throw switch may be used to select the circuit, whereas with the switches in the german-silver and nickel-silver lines it would be necessary to operate two switches to change from one circuit to the other. This would appear to be a

trifling detail, but in practice the advantage of complete control with one switch was found to be of considerable importance. The switches used in the copper lines must have blades and contact points of copper, otherwise the connections between the copper wires and the switch terminals might act as thermo-electric junctions and thereby invalidate the entire system. The use of brass screws to hold the copper wires to the copper terminals of the switch does not introduce thermo-electric junctions at these points, as the brass screws are not a part of the electrical circuit but serve merely in a mechanical way to hold the copper wires to the copper terminals.

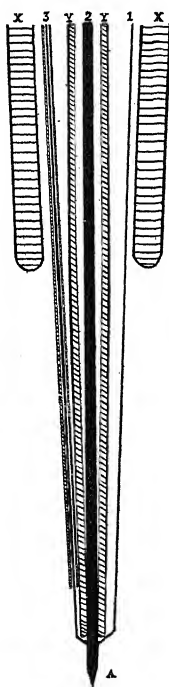


Fig. 2. Detail of construction of 3-wire thermo-electric junction. *A*, the 3-wire tip represented in fig. 1. 1, 40-gauge german-silver wire. 2, 28-gauge nickel-silver wire insulated from 1 and 3 by the glass tube *Y-Y*. 3, 40-gauge copper wire in a capillary glass tube. *Y-Y*, glass tube insulating and in part supporting the nickel-silver wire. *X-X*, end of the glass tube used to support the entire system leading to the tip *A*. Approximately $6\frac{3}{4}$ times actual size, except the opening of the tube *X-X* and the space shown between the various units, where a scale somewhat greater has been used.

The 3-wire junction A. As shown by figures 1 and 2, the 3-wire junction is built in the form of a 3-wire needle, the three wires being insulated from each other by glass tubing. Figure 2 shows a detailed sectional view of the tip of the 3-wire junction.

The mechanical support for handling the junction *A* is largely supplied

by the glass tube shown in part as $X-X$ in figure 2. This tube is drawn from a length of 4-mm. hard glass tubing, the last 8 cm. at the lower end tapering uniformly to a bore of 1 mm. at the open end. The length of the outer glass tube, of which only the end is shown in figure 2, is 34 cm.

The needle tip used to pierce the plant material consists of the sharpened end of the 28-gauge nickel-silver wire and the supporting tube $Y-Y$, as shown in figure 2. The glass tube $Y-Y$ extends the entire length of the 34-cm. outer tubing, serving both as a support for the tip and as insulation for the otherwise bare wire. The 40-gauge copper wire (no. 3, fig. 2), diameter about 80 microns, is carried the length of the tube $X-X$ in a capillary tube of 90 microns internal diameter. The glass in this case serves merely as an insulation for the otherwise bare copper wire, the tube being of little or no value as a mechanical support for the wire. The 40-gauge german-silver wire (no. 1, fig. 2), although bare, is not confined to a glass tube other than the outer glass tube $X-X$. As both the other two wires are glass-insulated, it is unnecessary to cover the third wire with an insulating tube. The capillary tubes for covering wires 2 and 3 are readily made from 4-mm. hard glass tubing by softening the glass in the blue flame of a Fisher burner until the proper degree of softness has been attained and then, with the tube removed from the flame, the glass is drawn rapidly or slowly according to the size tube desired.

The thermo-electric junction A consists of the union of the three wires as shown in figure 2. The 40-gauge copper wire and the 40-gauge german-silver wire are so placed as to meet the 28-gauge nickel-silver wire as nearly as possible at the same point. In figure 2 the two 40-gauge wires are shown as if meeting the 28-gauge wire from opposite sides of the tip A , whereas in reality the two 40-gauge wires meet the large (28-gauge) wire as nearly as possible at the same point, midway between the two contacts as shown in the figure. In this way the copper wire is in direct contact with both the german-silver and nickel-silver wires, and the two junctions thus formed are only a small fraction of a millimeter apart. The entire tip A , from the end of the supporting tube $Y-Y$ to the point, is dipped in molten, non-acid, radio solder, thereby soldering the two junctions. The capillary tube bearing the 40-gauge copper wire is attached to the side of the glass tube $Y-Y$ by a thin film of Dekhotinsky cement. The 40-gauge german-silver wire is likewise fastened to the side of the tube $Y-Y$. The open end of the tube $X-X$ is then sealed with this same cement. The entire assembly, from the end of the tube $Y-Y$ to a point about 12 mm. from the tip A , is then waterproofed by two thin coats of Duco (DuPont) paint. As a final preparation for use, the tip A is sharpened to a needle point by grinding on a very fine-grained oil stone.

At the upper end of the supporting tube $X-X$ the wires are securely fastened in place by sealing the end of the tube $X-X$ with a mass of Dekhotinsky cement. At this point, the upper end of the supporting tube $X-X$, the 40-gauge copper wire is soldered to a 14-gauge copper wire that leads to

one center terminal of the reversing switch *RS*. The three wires are then securely fastened to the side of the supporting tube by means of zinc oxide adhesive tape, such as is ordinarily used in surgical work.

A rubber stopper of the proper size to fit the opening of the freezing chamber to be used is then forced over the tube to a point about 12 to 14 cm. from the tip *A*. The entire length of the test junction assembly (the 3-wire junction and support) is about 35 cm. Smaller junctions of this same design could be made if so desired. The methods of making micro-thermocouples described by Whitaker (1929) could, with slight modifications, be used to make 3-wire junctions of this type should it be found desirable to construct even smaller junctions of this general design.

Construction of the reference junctions, B and C. The reference junction *B* (fig. 1) consists of the point of union of a 28-gauge nickel-silver wire and a 30-gauge copper wire. The two wires are kept from accidental contact by being led through glass tubes to the point *B* where the two wires are twisted together and soldered with a non-acid radio solder. At the upper end of the glass tube bearing the 30-gauge copper wire, the 30-gauge copper wire is soldered to a 14-gauge copper wire that leads to one end terminal of the switch *S*. The ends of the glass tubes are then sealed with Dekhotinsky cement and the two tubes, about 35 cm. in length, are fastened together at several points by this same cement. The reference junction *C* (fig. 1) consists of the union of a 40-gauge german-silver wire and a 30-gauge copper wire. Except that a 40-gauge german-silver wire is used instead of a 28-gauge nickel-silver wire, the reference junction *C* is constructed exactly like the reference junction *B*. The copper lead from this reference junction *C* is connected to the other end terminal of the switch *S*.

The two reference junctions, *B* and *C*, are placed through a single large glass tube of about 7 mm. internal diameter. The two junctions are arranged to extend about 3 cm. below the end of this tube and to extend not more than 2 or 3 mm. above the upper end of the tube. The lower end of the tube is then sealed with Dekhotinsky cement, thereby permanently fixing the relative position of the two reference junctions with respect to each other. The entire tip and all of the outer glass tube that is to be immersed in the ice-water bath are then coated with two liberal coats of Duco (DuPont) paint to seal any possible cracks in the cement, and to prevent the slow swelling of the Dekhotinsky cement which occurs when it is immersed in water for long periods of time. At the upper end of this outer glass tube the four lead wires are secured against abrasion by the glass edges by sealing the end of the outer tube with Dekhotinsky cement and then securing the four wires to the side of the tube by means of zinc oxide adhesive tape. The over-all length of this set of reference junctions is about 35 cm. The outer tube used to hold the two reference junctions has an external diameter of one centimeter, and a length of about 32 centimeters.

The entire reference junction assembly is then placed through a one-

centimeter hole in a rubber stopper, the stopper being of the proper size to fit the thermos bottle mentioned below. Through a second hole cut in the rubber stopper, a Heidenhain thermometer, calibrated to read at intervals of 0.01°C. , is inserted. The bulb of the thermometer is placed at the same distance below the stopper as the two reference junctions, and then the thermometer stem is securely fastened to the tube carrying these junctions by means of adhesive tape. For use, the entire assembly of reference junctions and thermometer is inserted into a one-quart vacuum thermos bottle filled with an ice-water bath at a temperature of 0°C.

The galvanometer. The galvanometer used with this 3-wire thermocouple system is a Leeds & Northrup No. 2284-X high-sensitivity galvanometer. The principal characteristics of this galvanometer, as stated by the manufacturers, are:

Sensitivity	0.033 microvolts per mm. at 1 m.
External critical damping resistance	15 ohms
Period	9.8 seconds
Resistance	16.1 ohms

Although the manufacturers of this instrument designed it primarily as a null-balance instrument for use with a high-sensitivity potentiometer, it has been found to be very useful as a deflecting galvanometer for use with thermocouple systems. With the 3-wire thermocouple system described in this paper, an external critical damping resistance of 15 ohms has been used only to check the swing of the galvanometer mirror when the circuit selecting switch (*S*, fig. 1) is opened, no damping resistance being used in series or parallel with either completed circuit of the thermocouple system.

In practice the scale and telescope were mounted at $\frac{1}{2}$ meter from the mirror of the galvanometer. The sensitivity of the system could, if desired, be increased proportionately by increasing the distance between the galvanometer mirror and the telescope and scale. The galvanometer was mounted on a concrete block resting on a laboratory table, the table being supported by steel legs and fastened to an inside wall. Both the floor and the inside wall were practically free from vibrations that would interfere with the proper working of so sensitive a galvanometer.

Other less sensitive and less expensive galvanometers could be used in connection with relatively more sensitive junctions than those described for use with this high-sensitivity galvanometer. The type of galvanometer required in any particular set-up would be dependent on the sensitivity of the junctions being used and the temperature range over which the system was to be used. The precision over two ranges, as described for the set-up in use by the writer, could hardly be duplicated in any set-up using a galvanometer of greatly reduced sensitivity unless thermocouple junctions of correspondingly increased sensitivity (increased thermo-electric power) were used. The practical difficulty in the construction of suitable junctions appears to be the limiting factor determining what other types of galvanometers could be used in

connection with 3-wire thermocouple systems of the general type described in this paper.

Calibration and use of the system. The 3-wire thermocouple system, for use, must be calibrated over the entire temperature range for which it is to be used. Such a calibration is most accurately made by placing the reference junctions in an ice-water bath at 0°C . and the 3-wire test junction in an alcohol bath at about -12°C ., using an accurately calibrated thermometer to check the temperature of this alcohol bath. Both the ice-water bath and the alcohol bath are kept in one-quart vacuum thermos bottles. When the temperature of the alcohol bath warms to the point at which the galvanometer deflection for the less sensitive circuit does not exceed the length of the scale, a temperature of -11.42°C . in this case, simultaneous readings of the thermometer and the galvanometer deflection are made. During the 10 to 15 hours required for the alcohol bath to rise from the temperature of -11.42°C . to 0°C ., several hundred readings of the thermometer and the corresponding galvanometer readings are taken. After the temperature is reached at which the galvanometer deflection for the more sensitive circuit does not exceed the length of the scale, a temperature of -3.125°C . in this case, 100 or more readings of the thermometer and galvanometer deflection are recorded for the more sensitive circuit, in addition to the readings being taken for the less sensitive circuit. It is well to obtain at least three such series of readings for each circuit. This involves several days' work, but it is only after very careful calibration that the system can be used to measure temperature differences to 0.001°C . with any degree of accuracy.

From the hundreds of readings for each circuit, a table of values to read in degrees centigrade can be made corresponding to the galvanometer readings for each circuit. The calibration values might also be plotted as a graph; however, to the present author at least, a table of values is preferable to a graph, as a table can more readily be read to 0.01° or 0.001°C . than can any graph of convenient size. When the calibration has been completed and the tables of values in degrees centigrade corresponding to the galvanometer readings have been prepared, the system may be considered as being ready for use in cryoscopic or other investigations.

Such a system should, in addition to being calibrated before use, be recalibrated at least once after having been in actual service for a period of time. In the present work, however, no significant difference in the calibration values could be found when the system was recalibrated after more than six months in actual service. Although this might lead one to conclude that the calibration values are practically constant, it would seem advisable to recalibrate the system from time to time during its use.

In using the thermocouple system to determine the freezing point depression values of living tissues, the size of the tissue mass being used must be considered. Theoretically the smallest block of tissue that could be used to determine the freezing point of that tissue would need to be of sufficient size

so that on freezing, after undercooling, sufficient heat would be liberated to raise the tissue and the thermocouple tip to the true uncorrected freezing point of that tissue, in opposition to the cooling effect of the surrounding air of the freezing chamber. Obviously, if the blocks of tissue used were too small, the heat liberated on freezing could not overcome the low temperature of the tissue and the junction and could not overcome the effect of the low temperature of the surrounding air of the freezing chamber. Under such conditions the observed freezing point would be too low and the value of the system would thereby be destroyed. In reality, no matter how large a block of tissue is used there will be a slight error due to the low temperature of the tissue and the thermocouple tip when the tissue freezes and the loss of heat by conduction from the tissue to the cold air of the freezing chamber. However, when a sufficiently large block of tissue is used to reduce this error below any significant value, this error may be disregarded. With this apparatus, as described, it has been found that above a certain minimum size of tissue block the freezing point depression value, corrected for undercooling, is independent of the size of the tissue block used and the extent of the undercooling before freezing commences. Below this particular minimum size of tissue block the corrected freezing point depression values vary widely with the size of the tissue block and the extent of the undercooling, the freezing point depression values in all cases being greater than for similar tissues cut in blocks above the minimum size. With the particular 3-wire thermocouple system described in this paper, blocks of tissue $3 \times 3 \times 2$ mm. seem to fulfill all the necessary conditions for overcoming the error due to the low temperature of the undercooled tissue and the effect of the low temperature of the surrounding air of the freezing chamber, while blocks of tissue $2 \times 2 \times 2$ mm. do not seem to fulfill these conditions. In practice, however, tissue blocks cut $3 \times 3 \times 3$ mm. or slightly larger, in some cases as large as $4 \times 4 \times 5$ mm., are used. As the time required to make a freezing point determination increases with increasing size of the tissue block, it is of practical value to use as small a block of tissue as is possible without sacrificing the accuracy of the determinations. The outstanding reason for using rather small blocks of tissue is that local variations and gradients in freezing point depression values can be measured.

In practice the tip of the 3-wire junction is forced into a block of the tissue, the elasticity of the tissue being sufficient to hold the tissue in place on the "tip." To prevent distillation of water from the tissue to the cold walls of the freezing chamber, the tissue is next coated with a rather thin layer of vaseline-oil mixture that readily congeals when the thermocouple tip with the tissue on it is inserted into the freezing air chamber.⁶ The tem-

⁶ The freezing air chamber used by the writer consists of an 18×150 mm. hard glass culture tube inserted into a 26×180 mm. brass tube which serves as a second air jacket. The brass tube, securely stoppered at its lower end, is suspended in an electrically refrigerated alcohol bath at about -30°C . The temperature in the inner air chamber—i.e., within the glass tube—is about -20 to -25°C .

perature of the tissue, as impaled on the thermocouple tip, suspended in the freezing chamber, falls rapidly (1 to 5°C. per minute, depending on the size of the tissue block) and soon passes below 0°C. The progress of this temperature drop may be followed from about 11.4°C. to -11.4°C. by timely operation of the reversing switch *RS*, shown in figure 1, the switch *S* being so closed as to complete the less sensitive circuit of the system. After the temperature falls below 0°C., the galvanometer deflection is continuously watched in order that the point of maximum undercooling may be observed and recorded. When the point of maximum undercooling is reached and ice crystallization (freezing) commences, the magnitude of the galvanometer deflection suddenly decreases, due to the rise in temperature of the undercooled tissue on freezing. When the deflection of the galvanometer thus decreases toward the zero point, the switch *S* is quickly reversed, thereby changing the system to the more sensitive circuit. In a very short time the galvanometer mirror temporarily comes to rest at the position corresponding to the freezing point of the tissue. This point is observed and recorded. The switch *S* is then opened⁷ and the zero point of the galvanometer (the point at which the mirror first comes to rest with the circuit opened) is observed and recorded. From these three readings the temperature of maximum undercooling and the uncorrected freezing point are found by referring to the tables of calibration values for the two circuits. The observed freezing point may then be corrected for undercooling in the same manner as if the two temperatures (maximum undercooling and observed freezing point) had been determined by a thermometer as ordinarily used in cryoscopic investigations. If it is then desired to study the effect of freezing at various temperatures on the subsequent freezing point of the tissue, the tissue may be frozen at the desired temperature, thawed, and again frozen without being removed from the thermocouple tip and without in any way disturbing the position of the tissue block on the tip.

SUMMARY

The purpose of this paper is to describe the construction and use of a 3-wire thermocouple system specifically designed for use in cryoscopic investigations on living plant tissues.

The use of thermocouple systems in cryoscopic work is briefly reviewed and discussed. A method of determining the freezing point depression values of plant tissues by means of thermocouples inserted directly into the tissues is described.

⁷ To check the rapid swing of the galvanometer mirror, the critical damping resistance switch (*CD*, fig. 1) is closed when the circuit selecting switch (*S*, fig. 1) is opened. This completes a circuit consisting of the galvanometer, wires 7 and 8, the switch *DS*, and the critical damping resistance coil (*CDR*) of 15 ohms resistance. The use of a critical damping resistance coil allows the galvanometer mirror to return rapidly to, but not beyond, its zero point without danger of too violent a swing damaging the suspension that holds the mirror and coil of the galvanometer. The critical damping resistance switch is finally opened as soon as the galvanometer mirror comes to rest.

Some of the advantages and disadvantages of the galvanometer and potentiometer-galvanometer methods of determining temperatures by means of thermocouple systems are discussed. The advantages and method of use of a 3-wire thermocouple system are fully discussed. The electrical hook-up of such a 3-wire system is described in detail and illustrated by two figures.

The construction of the 3-wire test junction and the two 2-wire reference junctions is discussed in detail. A satisfactory method of calibrating such a system is described.

A detailed description of the technique of using such a 3-wire thermocouple system in making freezing point determinations on plant tissues is given.

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LYCOPODIUM COMPLANATUM VAR. FLABELLIFORME
FERNALD: ITS ANATOMY AND A METHOD OF
VEGETATIVE PROPAGATION¹

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Lycopodium complanatum var. *flabelliforme* Fernald is the familiar "ground pine," found on juniper hillsides, on the outskirts of hemlock ravines, and in birch associations. It is a decorative ground cover, and is also used in making Christmas wreaths. It is not a rare plant, and is sold by nurseries. However, in the past, efforts to propagate it have proved singularly unsuccessful; the spores take eight years to develop to a mature gametophyte, and portions of the mature sporophyte transplanted from their natural environment or obtained from nurseries have generally failed to live. The work presented here was undertaken in an effort to discover the cause of this difficulty in the propagation of the mature sporophyte, and, if possible, to overcome it.

Very little literature has been found dealing especially with *Lycopodium complanatum* var. *flabelliforme* Fernald, although considerable work is available on other species of the genus. Russow (1872) and Jones (1905) present discussions of *Lycopodium complanatum* var. *flabelliforme* Fernald, Chamberlain (1932) mentions the similarity of the internal anatomy between root and stem (previously discussed in Russow's paper), and Campbell (1928) refers briefly to *Lycopodium complanatum* var. *flabelliforme* Fernald in connection with leaf arrangement and sexual organs. Valuable material about other species (many of which closely resemble *L. complanatum* var. *flabelliforme* Fernald) may be found in publications of Haberlandt (1914), Engler and Prantl (1902), Campbell (1928), Holloway (1909), Bower (1908), and Jones (1905). The last two named are particularly valuable, and in the introduction to Jones' paper is found a review of material on the genus published up to 1905.

ANATOMY

External. *Lycopodium complanatum* var. *flabelliforme* Fernald has a creeping main stem, lying on top of the ground or as far as one inch beneath the surface. The stem is covered with small bracts, and produces roots ventrally and shoots laterally. If the shoots remain upright, they bear the leafy portions; but if lying on the ground, or beneath it, they function as does

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the main stem, and give rise to both roots and leafy portions, as shown in figure 1 of plate 1. The root system is extensive, one main root and its branches occupying an area averaging four inches wide and five inches deep (pl. 1, fig. 1).

Internal. Much work has been done on the subject of the arrangement of the vascular tissue of the Lycopodiales. Bower (1908) shows clearly that "the vascular structure of the mature shoot is referable in origin in all cases to the non-medullated monostele . . . the modification has been by intrusion of the phloem more or less deeply into the xylem-core, till this may at last be divided into distinct plates. . . ." Examination of a cross section of the stem of *L. complanatum* var. *flabelliforme* Fernald shows this distinct plate-like division, with alternating bands of phloem and xylem, resulting in a complex protostele. These bands or plates constantly shift in shape and position, so that no two cross sections are identical. The protoxylem is peripheral, the cells being rather small and compact. The metaxylem is centrally disposed, its cells large and heavy-walled. There is no parenchyma in the xylem mass. Between the xylem bands, and separated from them by several rows of parenchyma cells, is the phloem, composed of sieve tubes and companion cells. The protophloem is peripheral, the metaphloem central.

It was found difficult to differentiate pericycle and endodermis. According to Bower (1908), the pericycle is the parenchymatous tissue immediately surrounding the xylem and phloem. This view is also held by Jones (1905), who states that the tissue is from two to six layers, narrower opposite the protoxylem and broader opposite the protophloem. Such a region was fairly easily located in cross sections of *L. complanatum* var. *flabelliforme* Fernald, and is referred to as pericycle in plate 1 (fig. 4, 5). The endodermis is clearly marked and heavily cutinized, according to Campbell (1928). Bower (1908) states that it is "obscured by the extension of the corky development." Jones (1905), in discussing *L. clavatum* (which is very similar to *L. complanatum* var. *flabelliforme* Fernald), says that the endodermis is the region of two to three layers of cells surrounding the pericycle region, "and stains very similarly to the xylem." However, he later speaks of the cuticularization of the endodermal walls. In cross sections of the stem of *Lycopodium complanatum* var. *flabelliforme* Fernald, a region of two to three layers of cells, larger and more regular in shape than the so-called pericycle cells which they encompass, could be seen, resembling almost exactly tissue which Jones (1905) labels endodermis in a plate showing a cross section of the stem of *L. clavatum* L. However, no cutinization of the walls could be detected (see microchemical study).

Jones (1905) states that the cortical region is composed of three zones: the innermost zone is very broad, some of the cells having "definite but not very obvious spiral thickenings"; the median zone is less sharply defined than in *L. clavatum* L., "and does not assume the appearance of water tissue"; the outer zone is reduced to one or two layers; the "cortical cells have their

long diameter approximately horizontal." Examination of cross and longitudinal sections seemed to indicate conditions not in accordance with all of Jones' observations. The so-called "innermost zone" showed definite annular rings and was composed of compacted, thick-walled cells. The cells of the median zone, distinctly parenchymatous in appearance and function (see microchemical study), when viewed in longitudinal section were not parallel to the main axis, but lay obliquely at an angle of about 20° with the vertical. (It should be noted that Jones used dried specimens sent from Germany.)

As Russow (1872) states, the anatomy of root and stem is identical; so the description is applicable to both (pl. 1, fig. 4, 5).

When stained with 0.5 per cent gentian violet in 5 per cent H_2SO_4 , thick strands of protoplasm are shown between adjacent tracheids of the xylem and adjacent parenchyma cells (pl. 1, fig. 3). Examination under the petrographic microscope shows strands between adjacent phloem cells. These strands are thin, while the strands between tracheids are grouped together and therefore appear as thick bands. This is not in accord with the statement made by Campbell (1928, p. 496): "According to Strasburger, the oblique end walls of the large tracheids show the same elongated pits as the lateral walls, but in no cases could any communication between adjacent tracheids be demonstrated."

MICROCHEMICAL STUDY OF CELL WALL STRUCTURE AND FOOD SUBSTANCES

All tests were made in accordance with those described in the unpublished outline on microchemical technique by Dr. Sophia H. Eckerson.

Epidermis. Treatment of a cross section of the stem with Sudan III shows a fairly heavy cutin layer on the external walls of the epidermal cells. The cutinization sometimes extends down the lateral walls and occasionally reaches the peripheral walls of the cells adjacent to the epidermis. When the cutin is dissolved out by means of 3 per cent alcoholic KOH, the walls give the reaction for cellulose with I-KI and 75 per cent H_2SO_4 .

The Flückiger test shows the presence of sugar stored in the cells.

Cortical parenchyma. The cell walls are cellulose. I-KI shows starch grains. The Flückiger test shows the presence of sugars, Cu_2O crystals appearing after warming at $50^{\circ}C.$ for thirty minutes. Millon's reagent shows protein crystals in the cell sap. Sudan III stains fat in globules and droplets throughout the cells.

Sclerenchyma. The tissue referred to as sclerenchyma is the region between the cortical parenchyma and the stele.

Alcoholic phloroglucin followed by 25 per cent HCl shows heavy lignification of the tissue. Ruthenium Red shows pectic substances in the tertiary cell wall.

There are a few fat droplets in the cells near the stele, and occasional starch grains.

Pericycle—endodermis. As shown in the anatomical study, the pericycle

and endodermis were not definitely located. The region of two to three layers of cells, fairly regular in shape and arrangement, lying immediately inside the sclerenchyma, does not give a positive test for cutin, lignin, cellulose, or pectic substances. The parenchymatous cells (probably pericycle) inside this region give a positive test for cellulose. This shows a chemical difference as well as a difference in shape and arrangement between the two regions. However, the facts that the outer region is several rows thick, and that the cell walls do not give the expected tests, leave some doubt as to whether it is a true endodermis.

Fat was observed in both regions.

Xylem. Protoxylem and metaxylem are heavily lignified. The middle lamella is clearly visible and indicated by the presence of pectinaceous materials.

Phloem. I-KI stains the walls a deep, dull blue. This might indicate the presence of amyloid. It is a good way to identify the cells. The addition of 75 per cent H_2SO_4 gives an immediate reaction for cellulose.

Fat is densely stored. Millon's reagent gives a positive reaction for protein, and sugars are shown to be present by the Flückiger test. Heating for at least 30 minutes at $50^\circ C$. is necessary to precipitate the Cu_2O crystals.

Parenchyma sheath between xylem and phloem. If sections are mounted in I-KI and observed in dim light under the microscope, the walls of these cells show a marked contrast to those of the adjacent phloem cell walls. The latter are blue, while the parenchyma cell walls are dark brown.

SUMMARY OF MICROCHEMICAL STUDY

Tissue	Wall substance	Food-substance
Epidermis	Cutin	Sugar
Cortical parenchyma	Cellulose	Sugar Starch Protein Fat
Cortical sclerenchyma	Lignin	Starch } Small Fat } quantities
Endodermis (?)	(?)	Fat
Pericycle (?)	Cellulose	Fat
Xylem	Lignin	
Phloem	Amyloid Cellulose	Fat Sugar Protein

ORIGIN AND DEVELOPMENT OF ROOT

Stokey (1907) showed the presence of "inner roots" in *Lycopodium pithyoides*. These roots are so termed because they are initiated at the apex of the stem, turn at a sharp angle, and bore through the cortex, sometimes for a distance of 25 cm., practically parallel to the stele of the stem. It was thought that possibly *L. complanatum* var. *flabelliforme* Fernald might also have this type of root, and, if so, a means of propagation would be suggested.

Longitudinal and cross sections failed to show the presence of these "inner roots." However, the sections showed roots in the main stem which had not emerged, but had pushed about half-way through the cortex. As contrasted with Stokely's "inner roots," these never turned and grew down through the cortex, but grew at right angles to the main axis of the stem. Their stele was not horseshoe-shaped, but similar to that of the stem. They were found at various points along the main stem, from the tip to as far back as 25 cm. from the tip. They could sometimes be located by small mounds which arose as they pushed through the stem, but often they were not sufficiently developed to give such a clue to their position. They were seen to originate from the pericycle region, as shown in plate 2 (fig. 1-7). This is in accordance with the work of Van Tieghem, as quoted in Campbell (1928). The name "arrested root" is given to them.

To determine whether these "arrested roots" had been initiated in the mature tissue, sections of many stems were made. Fully developed "arrested roots" were found, but never the very beginning of a root initial. However, at the tip of the stem ("tip" signifies that portion of the stem from the apex to 4 mm. back of it, as shown in pl. 1, fig. 1), several cases were found where root initiation was taking place. Forty cuttings of mature tissue were planted in peat. Twenty of these were cut so as to be composed of about two inches of main stem, with leafy shoot. Twenty were shoots cut at their exit from the main stem, since it had been shown that the shoot as well as the main stem could give rise to roots, as shown in plate 1 (fig. 1). Twelve cuttings were killed by a damping-off fungus. None of the surviving cuttings rooted. Ten runner tips were planted in peat. Two were killed by the same fungus. Within seven to ten days the others were examined, and roots were found about 4 mm. from the extreme tip. The runners continued healthy growth.

An examination of the initiation of the shoot was made. Shoot initials were found only at the tip of the main stem, originating from the pericycle region. Longitudinal sections showed that the shoot emerged from the stele of the stem, turned at a sharp angle and grew through the cortex, finally emerging 4 to 6 mm. from its point of origin (pl. 1, fig. 6a). As contrasted with the root, the shoot developed so that its vascular tissue was a parallel prolongation of the stem tracheary plates, whereas the root developed so that its vascular tissue was at right angles to the stem tracheary plates. (This is a means of differentiating between a very young root and shoot initial, seen in cross section.) The shoot emerged 4 to 6 mm. from its point of origin, whereas the root remained at right angles to the stem axis, and emerged where it originated (pl. 1, fig. 6a, 7a).

Since microscopical examination and experimental evidence seemed to point to the fact that there is no initiation of roots in mature tissue, but only at the stem tip, a comparison of the cell wall structure and cell contents in the two localities was made. Sugar, starch, fat, and protein were found in the

mature tissue (see summary of microchemical study). At the tip no fat or protein was observed. Sugar was present, and a quantity of starch. The starch grains were particularly concentrated in the cells surrounding the tissue which was differentiating into either a root or a shoot. Several serial sections of tips were made, which contained root initials. A lignin test was applied to these sections, but no lignin seemed to be present. In the mature tissue, the xylem and sclerenchyma were heavily lignified. An "arrested root" in mature tissue had lignified xylem, and the root tip was embedded in lignified sclerenchyma. The development of lignification is shown as follows:

1-4 mm. from stem apex	No lignin
5 mm. " " "	Protoxylem lignified
6 mm. " " "	Protoxylem lignified
		Sclerenchyma faintly lignified
7 mm. " " "	Protoxylem lignified
		Sclerenchyma lignified
		A few metaxylem cells lignified
10 mm. " " "	Protoxylem lignified
		Metaxylem lignified
		Sclerenchyma lignified

CONDITIONS THAT ARREST THE DEVELOPMENT OF THE ROOT

The cause of the formation of the "arrested root" was examined. Runners were planted under two contrasting conditions, some in contact with moist peat, others not in contact with moist peat. Under the moist conditions the runners rooted at their tips. Under the dry conditions no roots emerged, and sections of the new growth showed "arrested roots" to have formed. Runners with "arrested roots" (as indicated by mounds) were placed in contact with moist peat. After two weeks the roots emerged.

Casual observation seemed to indicate a fairly regular coincidence of root and shoot, indicating simultaneous stimulation for both. Since the shoot does not appear until an average of 5 mm. from its point of initiation (pl. 1, fig. 6a), it would be necessary to have the root appear about 5 mm. before the shoot, in order to prove any relation between their initiation. Examination was made of 161 cases. The following data were obtained:

Root 2-6 mm. before shoot	70	45 per cent
Root and shoot together	39	25 per cent
Root 6-10 mm. before shoot	5	3 per cent
Root after shoot	47	27 per cent

In other words, 45 per cent show the root emerging practically at the theoretical origin of the shoot, 28 per cent show the root emerging about 5 mm. before or after this theoretical origin, and 27 per cent show the root farther than 5 mm. from the shoot origin. The figures are not conclusive enough to prove any definite relation.

EXPERIMENTS ON PROPAGATION

The extensiveness of the root system was so striking, as shown in plate 1 (fig. 1), that it was thought that this might be one of the causes of the difficulty in transplanting rooted portions of the sporophyte. Accordingly, cut-

tings were taken which were composed of about three inches of main stem, a shoot, and as much as possible of the root system. Some soil was retained around the roots. Healthy growth continued. Figure 1 shows such a cutting. The light leaf to the left is the original foliage, and all the rest of the foliage developed from bud shoots on the original stem.



Fig. 1. Cutting of *Lycopodium complanatum* var. *flabelliforme* Fernald. The original foliage is on the left. The portion on the right developed from shoots.

When ordered from a nursery, long portions of main stem, with shoots and roots, are sent. The roots have obviously been pulled from the ground, and have not been packed in moss to keep them moist. Therefore they are often mutilated to the extent of having only the main root and a few secondary roots, which have dried up so much as to be practically useless. Since the tissue, being mature, does not form new roots, it is imperative to retain as much as possible of the existing root. When this care is not taken, the plant will not live. This seems to be one of the chief causes of the difficulty in transplanting portions of the mature sporophyte, obtained either from nurseries or from their natural environment.

It should be noted that in these experiments, some of the native soil was retained around the roots, and observations were carried on for only five months. There are, therefore, two points left unsettled: First, will the growth continue indefinitely? Second, is there an associated fungus in the native soil which is necessary for the life of the plant?

SUMMARY

1. Initiation of the root of *Lycopodium complanatum* var. *flabelliforme* Fernald is found only at the stem tip, where the tissue is meristematic and there is no lignification of the tissue. Cuttings of mature tissue do not root. An actively growing stem roots at the tip.

2. The root arises from the pericycle region, at right angles to the tracheary plates. It continues its development at right angles to the axis of the main stem, and does not form an "inner root."

3. If in contact with moist earth, the root will emerge at once. If not in contact with moist earth, it will remain in the stem, in which case its xylem becomes lignified, and the xylem and sclerenchyma of the stem become lignified also. This results in what we have termed an "arrested root."

4. The root system occupies an average area four inches wide and five inches deep, and in order to transplant, successfully, rooted portions of the sporophyte, as much as possible of the root system must be retained and kept intact during the transplanting.

5. Suggested means of propagation: (a) Stem cutting with root. Care must be taken to secure all of the root system and not merely the main root. (b) Runner tip. Regardless of whether the tip is already rooted or not, if well watered, growth at tip will continue and new roots will be laid down. (c) "Arrested root." This may be identified by a small mound on the under side of the stem.

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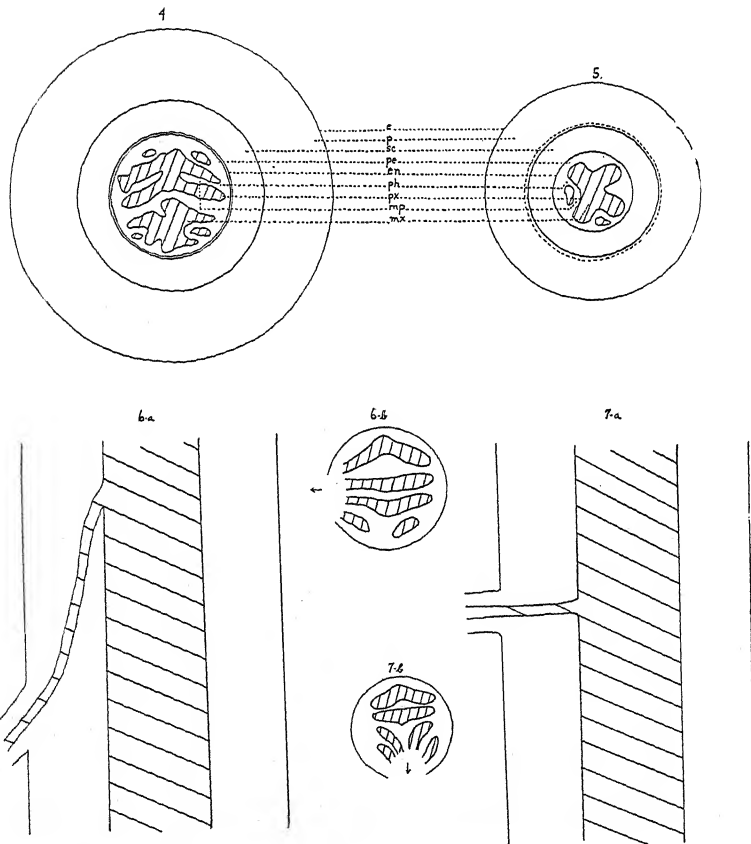
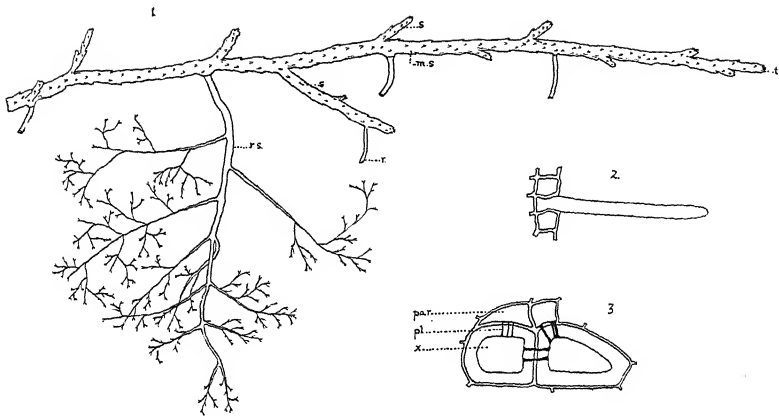


PLATE I. Fig. 1. Root and shoot; *r. s.*, root system; *s.*, shoot; *m. s.*, main stem; *t.*, tip of runner; *r.*, root. Fig. 2. Root hair. Fig. 3. Plasmodesma in stele of stem; *par*, parenchyma; *pl*, plasmodesma; *x*, xylem cell. Fig. 4. Diagram of stem. Fig. 5. Diagram of root; *e*, epidermis; *p*, parenchyma; *sc*, sclerenchyma; *pe*, pericycle; *en*, endodermis; *ph*, protophloem; *px*, protoxylem; *mp*, metaphloem; *mx*, metaxylem. Fig. 6a. Diagram of shoot leaving stem. Fig. 6b. Cross section of shoot. Fig. 7a. Diagram of root leaving stem. Fig. 7b. Cross section of root.

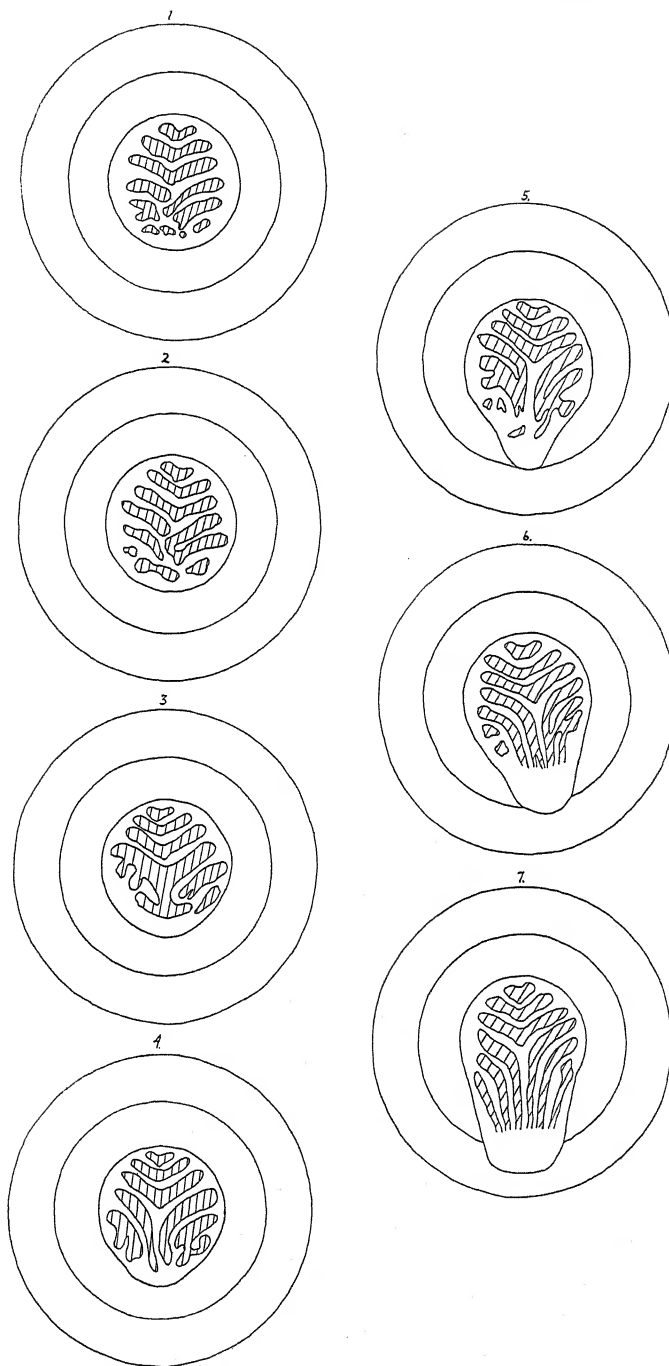


PLATE 2. Development of root from central stele of stem.

SOME NEW OR OTHERWISE IMPORTANT LABIATAE OF THE HAWAIIAN ISLANDS

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Haplostachys bryanii sp. nov.—Erecta, gracilis, forsitan 3–5 dm. alta, caule tetragono minute denseque incano-pubescenti pilulis plus minusve retrorsis. Folia tenuiter petiolata petiolis plerumque 1.5–3 cm. longis, laminis oblongis vel ovato-oblongis, basi cordatis sinu 2–4 mm. alto, apice obtusis, margine crenatis 10–24 dentibus pro unico latere, membranaceis, supra minutissime tomentulosi infra obscurissime venosis et incano-tomentosis, 2.5–4 cm. longis et 1.2–2 cm. latis. Inflorescentia simplex, spicata, \pm 2 dm. longa; bracteis oblongo-linearibus acutis 4–6 mm. longis vel inferioribus 1–1.8 cm. longis imis subfoliaceis. Calyx ad anthesin cylindraceo-obconicus, dense pubescens, 9–12 mm. longus, lobis angustis acribus 2–4 mm. longis. Corolla extrinsecus valde pubescens, tubo tenui 1–1.4 cm. longo, labiis margine crenulato-incisis crispisque superiore circ. 6 mm. longo inferiore paulo brevior 3-lobato lobis rotundatis.

Specimens examined: *George C. Munro* 553, Mauna Loa, western Molokai, June 1, 1916 (type, Herb. Bishop Mus.).

Named for Mr. Edwin H. Bryan, Jr., Curator of Collections, Bernice P. Bishop Museum, Honolulu. It was through Mr. Bryan's invaluable assistance in assembling for my study various materials (mostly unmounted and therefore not cited here) that the descriptions given here were made possible.

HAPLOSTACHYS BRYANII robusta var. nov.—Robustior, saltem 8 dm. alta, ramosior. Folia majora, petiolis tenuissimis 4–5.5 cm. longis, laminis ovato-cordatis, 5–7 cm. longis et 3.5–4.5 cm. latis sinu usque ad 7 mm. alto, dentibus majoribus non numerosioribus. Inflorescentia \pm 3 dm. longa.

Specimens examined: *Joseph F. Rock* 14000, Kalaeokalaau flats, southwesternmost Molokai (type, Herb. Bishop Mus.).

HAPLOSTACHYS BRYANII microdonta var. nov.—Gracilis, subsimplex, \pm 5 dm. alta. Foliorum petioli 2–3 cm. longi; laminis lanceolato-oblongis, 3.5–4.5 cm. longis et sub 1.8 cm. latis, dentibus parvis 25–36 pro unico latere.

Specimens examined: *Albert S. Hitchcock* 15139, in open, dry ground, western Molokai, Oct. 12, 1916 (type, Herb. U. S. Nat.).

In the species proper the teeth of the leaves average about 4–6 per centimeter, while in the var. *microdonta* they average about 9 or 10.

HAPLOSTACHYS GRAYANA angustifolia var. nov.—Folia infra etiam magis perspicue tomentosa reticulato-venosaque, principalia plerumque lanceolato-oblonga ac gradatim usque ad apicem saepius acutum angustata 5–10 cm.

¹ Published out of the order determined by the date of receipt of the manuscript.

longa sed tantum 1.5–3.2 (raro — 3.7) cm. lata. Corolla saepe paulo major, tubo usque ad 2.2 cm. longo, labiis usque ad 9 mm. longis.

Specimens examined: *Charles N. Forbes* 461H, slopes of Mauna Kea, Waiki, Isl. Hawaii, August, 1911 (Herb. Bishop Mus.; Herb. Mo. Bot. Gard.); *Jules Remy* 394, Isl. Hawaii, 1851–1854 (type, Herb. Gray: cotypes, Par., 2 sheets); *Joseph F. Rock* 8350, inner slopes of Crater Nohonaohae, plains of Waimea, Isl. Hawaii, June, 1910 (Herb. Bishop Mus.; Herb. Gray; Herb. N. Y. Bot. Gard.).

PHYLLOSTEGIA RACEMOSA bryanii var. nov.—A specie foliis paulo minoribus, lamina 1.5–3 cm. longa, verticillastris plerumque 6- raro 4-floris demum 1 cm. latis, floris minoribus, calyce 2–3 mm. longo, corollae tubo \mp 4 mm. longo differt.

Specimens examined: *Abbé Urbain Faurie* 908, Pukoo, Isl. Molokai, June, 1910 (Del.); *Dr. William Hillebrand*, heights back of Kamalo, Isl. Molokai (type, Herb. Berl.); *idem*, Mopulehu, Isl. Molokai, 1870 (Herb. Berl. et Herb. Kew, cum *P. hispida commixta*; Herb. Gray).

Named for Mr. Edwin H. Bryan, Jr., Curator of Collections at the Bernice P. Bishop Museum, Honolulu, to whom I am indebted for the privilege of examining certain valuable specimens.²

PHYLLOSTEGIA FLORIBUNDA forbesii var. nov.—Caulis infra sparsim supra dense brevi-hispidus setulis minutis erecto-adpressis. Folia supra sparsim ac breviter adpresso-hispida, infra secundum venas subadpresso-pubescentia alibi glabrata sed numerosissime ac minutissime resinoso-punctulata. Inflorescentiae pili breviores, saepe glanduloso-capitati; calyce dimidio minore, valde resinoso-glanduloso.

Specimens examined: *Charles N. Forbes* 294H, Kaalapuuwale, Kona, Isl. Hawaii, June 30, 1911 (type, Herb. Mo. Bot. Gard.).

PHYLLOSTEGIA HELLERI imminuta var. nov.—Caulis ramique subtiliter denseque pubescentes. Folia basi rotundata vel truncata raro subcordata, supra sparsim hispida setulis brevissimis, infra molliter pubescentia ac minutissime plus minusve glandulosa. Verticillastra minora, demum \mp 1 cm. lata. Calyx paulo minor, demum plerumque sub 3 mm. longus.

Specimens examined: *Charles N. Forbes* 21L, Kaiholena Valley, Isl. Lanai, June, 1912 (Herb. Bishop Mus.; Herb. Field Mus.); *idem* 63L, mountains near Koele, Isl. Lanai, June, 1913 (Herb. Bishop Mus.); *Mrs. Charles N. Forbes*, Kaiholena, Isl. Lanai, Mar. 17, 1916 (type, Herb. Bishop Mus.); *George C. Munro*, eodem loco et tempore (Herb. Field Mus.); *idem* 96, eodem loco, Sept. 16, 1913 (Herb. Bishop Mus.); *idem* 99, eodem loco et tempore (Herb. Bishop Mus.); *idem* 490, Isl. Lanai (Herb. Bishop Mus.; Herb. Field Mus.); *idem* 680, Kalulu, Isl. Lanai, Apr. 2, 1919 (Herb. Bishop Mus.; Herb. Field Mus.).

In habit closer to *P. mollis* Benth., but the fruiting calyces betray at once a strong affinity with *P. helleri*.

PHYLLOSTEGIA BREVIDENS var. longipes (Hillebr.) comb. nov.; *P. ambigua* var. *longipes* Hillebr. Fl. Haw. Isls. 350. 1888.

PHYLLOSTEGIA BREVIDENS var. hirsutula (Hillebr.) comb. nov.; *P. grandiflora* var. *hirsutula* Hillebr. loc. cit. 349.—The type (Herb. Berl.) has the

² Unmounted and hence not cited here.

hairs on its raceme's axis distinctly spreading or suberect, as is characteristic of *P. brevidens*, and not retrorsely appressed as in *P. grandiflora*, to a variety of which Hillebrand referred it.

PHYLLOSTEGIA BREVIDENS heterodoxa var. nov.—Rami supra adpresso-hispiduli, infra glabrati. Folia supra glabrata sed infra moderate adpresso-hispida, plerumque ovato-oblonga, basi late cuneata vel truncata, 10–13.5 cm. longa et 5–7 cm. lata. Verticillastra 10–12-flora, pedicellis 7–13 mm. longis; calyce adpresso-hispido, 6–7 mm. longo, lobis 0.2–0.4 longitudinis tubi; corolla externe plus minusve hispida, parva, tubo tantum circ. 7–8 mm. longo, labio inferiore quam tubo non vel saepe longiore.

Specimens examined: *Joseph F. Rock* 10014, Naalehu, Kau, Isl. Hawaii (type, Herb. Bishop Mus.: cotype, Herb. Field Mus.).

PHYLLOSTEGIA MOLLIS lydgatei var. nov.—Inflorescentiae rami elongati internodiis saepe 2–4 cm. longis, verticillastris plerumque 6-floris, pedicellis patenti-hispidis (nonnullis setulis saepe glandulosis) et 4–7 mm. longis. Calyx major, numerose ac subdistincte nervius, breviter hispidulus, minutissime glandulosus, acriter tenuiterque lobatus, 5–6 mm. longus; corollae tubo circ. 8–9 mm. longo.

Specimens examined: *Dr. William Hillebrand*, Isl. Oahu (Herb. U. S. Nat.); *Rev. J. M. Lydgate*, Waianae Mts., Isl. Oahu, 1869 (type, Herb. Berl.); *idem*, Makaleha, Isl. Oahu (Herb. Berl.).

This, like the var. *glabrescens* Deg. and Sherff, has calyx lobes suggestive of *P. stachyoides* A. Gray, but the general habit is distinctly that of *P. mollis*.

PHYLLOSTEGIA MOLLIS micrantha var. nov.—Inflorescentia ramosior, ramis brevibus (\approx 5 cm.), verticillastris confertis, pedicellis sub 1 mm. longis. Calyx circ. 2 mm. longus, lobis triagulatis, acutis, brevibus; corolla minuta, tubo 3–5 mm. longo.

Specimens examined: *Charles N. Forbes* 170L, Maunalei Valley, Isl. Lanai, June, 1913 (type, Herb. Bishop Mus.).

PHYLLOSTEGIA STACHYOIDES hitchcockii var. nov.—Folia infra secundum venas adpresso-pubescentia aliter glabrata sed glandulis numerosissimis minutis atris punctulata. Pedicellae suberecte pubescentes, inter pilulos paucis glandulis saepe atro-punctulatae, circ. 2–4 mm. longae. Calyx perspicue atro-punctulatus, circ. 5 mm. longus.

Specimens examined: *Albert S. Hitchcock* 15069, in rain-forest, Mr. Conradt's place, Pukoo, Isl. Molokai, Oct. 8, 1916 (type, Herb. U. S. Nat.).

PHYLLOSTEGIA PARVIFLORA canescens var. nov.—Pubescentia subtilior breviorque. Folia membranaceissima, venis minus prominentibus, faciei inferioris pilulis tenerrimis et plerumque tantum secundum venas dispositis. Pedicellae 6–9 mm. longae.

Specimens examined: *U. S. S. Pacif. Explor. Exped. under Capt. Wilkes*, on the mountains of West Maui, 1840 (type, Herb. U. S. Nat.: cotype, Herb. Gray).

PHYLLOSTEGIA PARVIFLORA major var. nov.—A specie pedicellis paulo robustioribus, 11–15 mm. longis, calycibus 4–5 mm. longis differt.

Specimens examined: *H. F. Bergman*, alt. 1700 ft., in wet soil on shaded slope near Kaauumakua, Isl. Oahu, Feb. 22, 1928 (Herb. Bishop Mus.); *Charles N. Forbes*, Punaluu Mts. between Punaluu and Kaipaupau, Isl. Oahu, Nov. 14–21, 1908 (Herb. Bishop Mus.);

idem 1306O, valley and ridge east of main Konahuanui trail, Isl. Oahu, Apr. 16, 1909 (Herb. Bishop Mus.); *Joseph F. Rock* 565, Koolauloa Mts., Punaluu, Isl. Oahu, Nov. 14-21, 1908 (type, Herb. Bishop Mus.); *idem* 566, *eodem loco et tempore* (Herb. Field Mus.); *idem* 17313, Hawaiian Isls. (Herb. Bishop Mus.); Nobue Tsuji, Kahana, Koolauloa Mts., Isl. Oahu, Oct. 16, 1932 (Herb. Bishop Mus.).

The plants examined offer an illusory resemblance to *P. brevidens*, but the numerous glands of the inflorescence easily separate them from that species and its varieties.

STENOGYNE CALAMINTHOIDES waimeana var. nov.—A specie foliis infra dense tomentoso-hispidis differt.

Specimens examined: *Charles N. Forbes* 478H, Kohala Mts., Waimea, Isl. Hawaii, Sept. 7, 1911 (type, Herb. Bishop Mus.: cotype, Herb. Mo. Bot. Gard.); *Albert S. Hitchcock* 14369, Waimea, Isl. Hawaii, Aug. 26, 1916 (Herb. U. S. Nat.); *Joseph F. Rock* 8307, Hawaiian Isls. (Herb. Gray).

STENOGYNE CALAMINTHOIDES oxyodonta var. nov.—A specie calyce 1-1.4 cm. longo lobis plerumque acutis atque oblongo-lanceolatis vel subulato-lanceolatis fere vel satis tubo aequalibus differt.

Specimens examined: *Joseph F. Rock* 8379, Hawaiian Isls. (type, Herb. Bishop Mus., 2 sheets); *idem* 8381, *eodem loco* (Herb. Bishop Mus.).

STENOGYNE SCROPHULARIOIDES nelsonii (Benth.) comb. nov.; *S. nelsoni* Benth. in DC. Prodr. 12: 1848; Labiat. 655. 1835; *S. scrophularioides* var. β , A. Gray, Proc. Amer. Acad. 5: 347. 1862.

STENOGYNE SCROPHULARIOIDES biflora var. nov.—Folia numerosa, quam internodia longiora; lamina rotundato-ovata, basi subcordata, apice obtusa, supra sparsim infra paulo densius adpresso-hispida, 2.5-3.7 cm. longa et 1.8-2.8 cm. lata. Verticillastra biflora, pedicellis pubescentibus. Calyx usque ad 1.2 cm. longus, lobis plus minusve obtusis. Corolla non visa. Achænia oblongo-cuneata, apice irregulariter corniculata, circ. 4 mm. longa.

Specimens examined: *Dr. William Hillebrand* 342, Isl. Hawaii (type, Kew).

Stenogyne glabrata (Hillebr.) comb. nov.; *S. rotundifolia* var. *glabrata* Hillebr. Fl. Haw. Isls. 361. 1888.

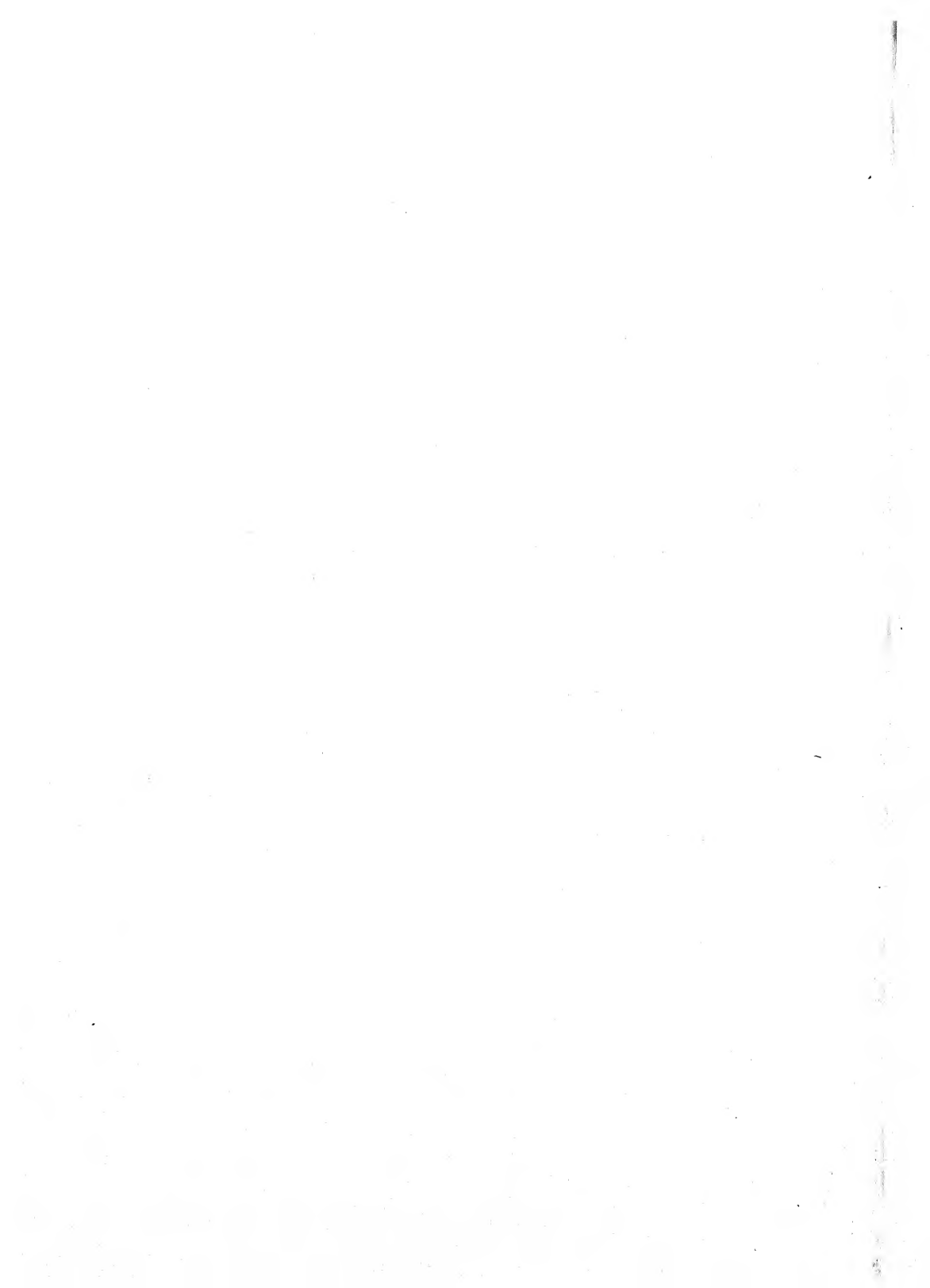
STENOGYNE ROTUNDIFOLIA oblonga var. nov.—A specie foliis majoribus, petiolo usque ad 2.5 cm. longo, lamina oblonga vel oblongo-ovata 4-5.5 cm. longa et 2-3.3 cm. lata differt.

Specimens examined: *Charles N. Forbes* 1112M, Kaupo Gap, crater of Mt. Haleakala, East Maui, Aug. 10, 1919 (type, Herb. Bishop Mus.: cotypes, Herb. Field Mus.; Herb. Kew).

STENOGYNE PURPUREA forbesii var. nov.—Foliorum laminae ovato-lanceolatae vel ovatae, infra molliter ac dense pubescentes; calyce maturo tomentoso.

Specimens examined: *Brodie*, Isl. Kauai, 1909 (type, Herb. Bishop Mus.); *Charles N. Forbes* 191K, Wahiawa Mts., Isl. Kauai, August, 1909 (Herb. Bishop Mus.; Herb. Mo. Bot. Gard.); *Rev. J. M. Lydgate*, *eodem loco* (Herb. Bishop Mus.).

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ABSTRACTS OF THE PAPERS PRESENTED BEFORE THE PHYSIOLOGICAL SECTION OF THE BOTANICAL SOCIETY OF AMERICA, PITTSBURGH, PA., DECEMBER 27-29, 1934¹

A Study of Irritability in the Plant. *W. E. Burge and G. C. Wickwire, University of Illinois, Urbana, Ill.*—The object of this paper is to raise a question concerning the method used by plant physiologists for determining different degrees of irritability. *Mimosa pudica* is the plant most frequently used for such work, and the extent of movement or drop of the leaf has been taken as a measure of the degree of irritability. Anything that increased the extent of drop of the leaf has been considered to increase irritability and vice versa. In animal physiology the height of contraction of a muscle is not a measure of the degree of irritability but of contractility. Irritability is the power of living material to respond to stimuli; muscle responds by contracting, the gland by secreting, and *Mimosa* by movement or drop of its leaf. Suspending a weight from a leaf of *Mimosa* decreases the extent of the drop of a leaf when it is stimulated, but we find it does not decrease its irritability. That is, the weight does not decrease the power of the leaf to respond to the stimulus, although it does decrease the extent of the drop of the leaf. Increasing the strength of stimuli, on the other hand, increases the extent of the drop of the leaf of *Mimosa*, according to Bose, but the stronger stimuli probably do not increase irritability, but rather decrease it, as is shown by a quicker production of fatigue when irritability sinks to zero with the use of the stronger stimuli. It seems to us that all of the large amount of work that has been done with *Mimosa* by Bose and other investigators where extent of drop of the leaf has been used as a measure of the degree of irritability has, in reality, not been on irritability at all.

Further Study on "Frenching" in Orange Trees. *G. C. Wickwire and W. E. Burge, University of Illinois, Urbana, Ill.*—Analyses made for several inorganic elements of samples of earth taken from different parts of a Florida orange grove showed, where the trees were not frenched, the presence of manganese and small amounts of iron and, where the trees were frenched, no manganese and large amounts of iron. The use of manganese apparently

¹ Printed and distributed in advance of the regular issue, at the expense of the Physiological Section. November 30, 1934.

remedies the condition just as the use of copper had been found to do, although no quantitative determinations of the chlorophyll of the leaves of the trees which were treated with manganese have as yet been made, as was done after treatment with copper.

Sand Culture of Seedlings as Compared with Soil Culture. *A. A. Dunlap, Connecticut Agricultural Experiment Station, New Haven, Conn.*—A variety of seedlings have been successfully cultured in pure sand to which nutrient solution had been added. Germination and growth of the seedlings in the sand were found to be equally as good as in the soil. Emergence and survival were frequently better in the sand. Seedlings grown in sand had well-developed root systems and were readily transplanted. The sand cultures were free from growth of parasitic fungi, even when the moisture content was sufficient to produce maximum growth of the seedlings.

Differential Distribution of Auxin in the Growing Leaf of Tobacco. *George S. Avery, Jr., Connecticut College, New London, Conn.*—The concentration of the hormone in the tobacco leaf is, roughly, inversely proportional to the age of the leaf—that is, it is present in greater concentration in young leaves and tends to disappear as a leaf matures. The midrib and main lateral veins are centers of accumulation and transport. While it is distributed approximately equally over the mesophyll and smaller veins of the entire lamina, polar transport and subsequent accumulation in the basal portion of the leaf obscure this effect. There is some evidence that in different portions of the leaf differential growth intensities are correlated with a differential distribution of auxin. While it is almost certain that the growth of the petiole depends on auxin—and possibly the midrib and main lateral veins also—there is no evidence that it is the limiting factor in the enlargement of mesophyll.

The Photoelectric Movements of *Mimosa Pudica* in Relation to Intensity and Wave-Length. *Paul R. Burkholder and Robertson Pratt, Connecticut College, New London, Conn., and Columbia University, New York City.*—Seedlings of *Mimosa pudica* were dark-conditioned to close the leaves during the daytime, and then were tested for the rate of leaf opening when subjected to radiation of measured intensity and controlled wave-lengths. Upon exposure to light from a 1000-watt tungsten lamp, the rate of leaf opening in dark-conditioned plants was dependent upon the intensity of radiation, opening being more rapid at higher intensities. Over the radiation range 2.2 to 417.0 ergs/mm.²/sec., the time required for opening may be described as a hyperbolic function of the intensity. In radiation of sufficient intensity (obtained from a tungsten lamp and a quartz mercury arc) up to values of about 55 ergs/mm.²/sec., leaf opening occurred readily in blue to long ultra-violet and in long red radiation; but little or no response was evoked by orange, yellow-green, or infra-red radiation. The suitability of *Mimosa* for the further investigation of certain problems is suggested.

Studies on the Photeolic Movements of *Mimosa Pudica*. *Paul R. Burkholder and Robertson Pratt, Connecticut College, New London, Conn., and Columbia University, New York City.*—Seedlings of *Mimosa pudica*, grown in a greenhouse maintained at 24°–28°C., were subjected to experiments designed to measure the response of the leaflets to darkness and to light at different times of day and night. The experimental temperature was maintained at 27.5°–29.5°C. Photic excitability in *Mimosa pudica* was found to be high and fairly constant during the daytime, as indicated by the alternate opening and closing of the leaves in light and darkness. The plants were unresponsive to light for a period of several hours after the normal closing in the evening, but photic sensitivity was regained early the following morning. The diurnal photeolic rhythm could be altered by changing the periods of light and darkness. Since “light and dark adaptation” differ from mechanical stimulation in the time required for response and recovery, it is suggested that the intracellular phenomena may be different in the two cases. The increment of the internal state of “dark adaptation” was studied by adapting plants to darkness in a graded series of dark periods and then measuring the time required for recovery in subsequent illumination; “dark adaptation” was accomplished within 20–30 minutes. An intracellular, amphoteric mechanism is suggested to account for the reversible responses of the pulvinus tissue concerned in photeolic leaf movements.

Further Studies on the Effect of Ultra-Violet Radiation on Seedlings. *H. W. Popp and Florence Brown, Pennsylvania State College, State College, Pa.*—In continuation of previous studies, the authors, during the past year, have exposed germinating seeds to selected regions of the spectrum of a mercury vapor lamp under equalized total energies. This was accomplished by determining the total energy transmissions of the screens used and adjusting the distances from the lamp. Screens were selected which made it possible to compare effects of ultra-violet alone, visible and ultra-violet, and infrared alone. The results in general agreed with those of former tests. Determinations of fresh and dry weights and chemical analyses of carbohydrate and nitrogen content were also made. There was a marked decrease in percentage of reducing sugar in plants exposed to the full spectrum of the mercury vapor lamp.

Glutathione and Sulphate in the Potato Tuber. *John D. Guthrie, Boyce Thompson Institute for Plant Research, Inc., Yonkers, N. Y.*—Ethylene chlorhydrin treatments of freshly harvested potato tubers increase the glutathione content and decrease the sulphate content. The decrease in sulphate is probably due to sulphate being used in the synthesis of glutathione. Analytical results show that 60 per cent of the decrease in sulphate is accounted for by the increase in glutathione.

Diurnal Change in the Citric Acid Content of the Leaves of *Bryophyllum calycinum*. *John D. Guthrie, Boyce Thompson Institute for Plant Research, Inc., Yonkers, N. Y.*—The diurnal change in the acidity of the juice of the leaves of *Bryophyllum* and other succulent plants is usually assumed to be due to a change in malic acid. Analyses for citric acid by the pentabrom-acetone method show that there is a five-fold increase in citric acid during the night and that this change accounts for 25 per cent of the change in total acidity.

Partial Inhibition of Photosynthesis and Reduction of Growth of Plants and Suppression of Remote Ancestral Characters Removed by Hybridizing with Remotely Related Species. *Walter T. Swingle, U. S. Department of Agriculture, Washington, D. C.*— F_1 hybrids between very different species of trees sometimes show extraordinary energy of growth, which implies a much more efficient use of solar energy in photosynthesis and of the inorganic food absorbed from the soil water than either of the parent species shows. Such extraordinary growth is shown by the so-called Paradox walnut, a hybrid of the Persian walnut (*J. regia*) of central Asia and the black walnut (*J. Hindsii*) of California. Both of these are among the largest and fastest growing species of the *Juglans*; yet the Paradox hybrid shows a rate of growth in California more than 10 times greater than the mean of the growth of the two parent species. This phenomenal heterosis can be explained if we assume that factors which inhibit photosynthesis and efficient utilization of inorganic foods are prevented from exerting their full action, because the two species of *Juglans* have developed widely different inhibitory mechanisms which are mutually antagonistic. The blocking of inhibitory action leaves the plant free to utilize to the full the sun's energy and the food absorbed from the soil. The remote ancestors of *Citrus* and the very distinct but related genus, *Poncirus*, undoubtedly both had, millions of years ago, odd pinnate leaves with opposite leaflets, but never develop them now. Hybrids of these genera, however, not infrequently show occasional beautifully formed pinnate leaves. This reappearance of these remote ancestral characters is probably due to the conflict of radically different mechanisms developed by *Citrus* and *Poncirus* for suppressing these characters resulting occasionally in a complete block of inhibitions, permitting the development of leaves of the remote ancestral type.

The Accumulation of Ions: Relation Between Protoplasm and Sap in *Valonia*. *S. C. Brooks, University of California, Berkeley, Cal.*—The distribution of Rb between sea water to which RbCl had been added and the sap and protoplasm-cell wall residue of *Valonia ventricosa* was determined at intervals after immersion of the coenocyte in the sea water solutions. Rb appears to collect in extremely high concentrations in the protoplasm during the first 2 days or less, and thereafter to pass not only on into the vacuole but also

back into the sea water, possibly in exchange for Na. Theoretical implications are discussed.

The Physiological Reactions of Forage Crops Under the Various Methods of Field Curing. *T. N. Jones and L. O. Palmer, Mississippi Agricultural Experiment Station, State College, Miss.*—Alfalfa windrowed as cut, and 2 hours after cutting, showed better color, quality, and less shattering of leaves than hay cured in the swath. The double windrow was better in this respect than the single, and hay double windrowed 2 hours after cutting lost a larger percentage of moisture during the first day than did hay in any other position. Photomicrographs of stomata on the leaves show that they remain open longer in windrows than in the swath and that they tend to reopen when windrowed 2 hours after cutting. This is in accordance with the more rapid rate of moisture loss from hay in this position and may be a possible explanation of this phenomenon. Leaves of both alfalfa and Johnson grass aid materially in the removal of water from the stem as revealed by several tests on the rate of moisture loss from plants with leaves attached, and with leaves removed. Moisture loss from plants with leaves remaining intact exceeded loss from stripped plants at the end of a 10-hour period by 5 per cent in the case of alfalfa and 3 per cent in the case of Johnson grass; but withering of thin alfalfa leaves prevented such a margin after 20 hours, while thicker leaves of Johnson grass supported by the fleshy midribs constantly increased the margin even up to 30 hours after cutting.

The Present Status of the Plasmodesmata Problem. *L. G. Livingston and I. W. Bailey, Harvard University, Cambridge, Mass.*—The numerous controversial points and divergences of opinion relative to the occurrence and true nature of plasmodesmata in plant tissue are largely due to the lack of a critical method for their study in an unaltered condition. The usual swelling techniques employed in their demonstration bring about profound alterations both in the cell wall proper and in the plasmodesmata present in the wall. Dehydration techniques bring about equal if not greater distortion of unlignified primary walls than properly controlled swelling. The only way to arrive at a satisfactory understanding of the true nature of plasmodesmata in the living plant is to study the cell walls in an unaltered state. In the studies here reported, it has been possible clearly to demonstrate and study plasmodesmata in untreated sections cut from the living cambium of *Pinus strobus* in the winter condition. In this tissue, plasmodesmata are found to be universally present in all primary pit areas. The threads penetrating the walls from adjacent cells are intimately associated with one another if not actually continuous. The evidence available indicates that they are protoplasmic in nature. They are extremely minute structures, the diameter of the thread itself in *Pinus* not being much more than $0.2\ \mu$. Preliminary observations on

other plants would indicate that plasmodesmata are generally of the same magnitude in the various tissues of higher plants. It is felt that reports of the presence of very large protoplasmic processes, several microns in diameter, interconnecting the cells of higher plants, are due to the lack of critical observation of the wall in the region of the primary pit areas.

A Simple Apparatus for the Laboratory Demonstration of Photosynthetic and Respiratory Ratios. *B. S. Meyer and Don S. Rader, Ohio State University, Columbus, Ohio.*—The apparatus described is suitable for demonstration purposes, and for laboratory practice by students of plant physiology. Values for the photosynthetic ratio of 1.00 ± 0.05 can be obtained with this apparatus for the leaves of various species with only a modicum of skill. The respiratory ratios typical of various plant materials can also be demonstrated quantitatively.

Yarovization of Winter Barleys. *Dmitry N. Borodin, U. S. Department of Agriculture, Washington, D. C.*—Fifty strains of barleys belonging to four botanical species from the World collection, including forms from India and Himalayas, were treated for producing yarovization effect. The response to the pre-sowing treatment, or yarovization formulas, used is specific for any one strain. Under the term "yarovization formula" is understood the predetermined combination of three factors: moisture (with or without chemical retarders), time, and temperature, indicated concisely in a ratio of "A:B:C," in which "A" is moisture percentage per dry weight of seeds, "B" time of treatment in days, and "C" temperature in degrees centigrade. Roman figures after "A" (in parentheses) represent the type of chemical retarder "R." Four yarovization formulas were used, namely: 50 (R.I.):28:5; 50 (R.I.):62:5; 50 (R.I.):62:2; and 50 (R.I.):62:10. The first formula failed to produce yarovization effect in pot experiments. Three others were applied in accordance with observations of the embryonic growth of seeds. Out of fifty strains of treated barleys, seven produced typical yarovization effect, or "winter type response" (full heading in experiment and grass cluster in check), ten by acceleration of heading and ripening, or "winter-spring" type response, and two by slight acceleration of heading or "spring-winter" type response. Thirteen strains did not respond to the treatment used, remained in the grass cluster stage both in experiment and check, and were classed as "stubborn winter type." Nine varieties produced heads in both experiment and check, or "spring type response." The yarovization effect among barleys is more diversified than in the case of winter oats.

Diurnal Fluctuation and Water Deficit Shrinkage in Thickness of Pineapple Leaves. *Maurice B. Linford, University of Hawaii, Honolulu, T. H.*—The pineapple plant is notably unresponsive to drouth, giving visible

indications of soil moisture deficiency or of root disease only tardily. Its mature foliage leaves possess a functioning water-storage tissue of palisade-like cells beneath the adaxial epidermis, varying in thickness but usually constituting over 25 per cent of total thickness in a median position. Successive measurements at marked points in a middle position on seven leaves per plant, made with a machinist's micrometer caliper in experienced hands, serve to detect as significant (odds over 100:1) changes as slight as 0.03 mm. This approximates 1.7 per cent of the thickness of an average leaf. Upon desiccation of a plant, either from depletion of soil moisture or from excision of roots, leaves may become 20 to 35 per cent thinner prior to an onset of color changes or drying of leaf tips. This is associated with collapse of the water tissue, and involves the loss of 15 to 20 per cent of the weight of a non-fruiting plant. Plants with roots intact, when irrigated copiously, may replenish their water store and regain their normal leaf thickness within 4 days. Control plants with sound root systems in moist soil exhibit irregular diurnal fluctuation in leaf thickness as great as 0.07 mm., or 4 per cent, measured in a median position. Subject to irregularities from day to day, shown similarly by parallel plants, thickness is characteristically greatest between noon and 4 p.m. and least in early morning.

The Heavy-Water Effect in *Spirogyra* in the Absence of Light. *T. Cunliffe Barnes, Yale University, New Haven, Conn.*—Most investigations on photosynthesis have dealt with the CO₂ molecule, the chlorophyll concentration, or the end products, while the most important factor—the type of water molecule utilized—has been ignored. The author has shown that *Spirogyra* filaments live longer in 0.5 per cent heavy water than in ordinary water (Moore's solution added in each case). The present report deals with differences observed when these experiments are repeated in the dark. The results will serve as a basis for a brief discussion of the possible isotopes and polymers in water utilized in photosynthesis.

The Experimental Production and Physiological Characteristics of Hermaphrodite and Monoecious Plants in Certain Normally Dioecious Species. *W. F. Loehwing, State University of Iowa, Iowa City, Iowa.*—A description is given of methods of inducing experimentally the functional development of staminate and pistillate primordia in the same flower of several normally dioecious species. Sex ratios among the progeny of self- and cross-pollinated parents are given, as well as the physiological differences between normal unisexual and experimentally produced bisexual plants.

The Action of Pectic Solvents Upon Cotton Fibers. *Wanda K. Farr, U. S. Department of Agriculture, stationed at Boyce Thompson Institute for Plant Research, Inc., Yonkers, N. Y.*—Treatment of mature, finely cut cotton fibers with solutions of 2 per cent ammonium oxalate or ammonium citrate

for $\frac{1}{2}$ to 1 hour, filtration, and the addition of 2 to 3 cc. of hydrochloric acid to the filtrate resulted in the formation of large quantities of a flocculent, yellowish-gray precipitate. Similar precipitates were formed, in varying quantities, by treatment for 1 hour with 1 per cent oxalic, citric, hydrochloric, tartaric, malic, and sulphuric acids followed by the addition of alcohol. Two per cent solutions of potassium and sodium hydroxides were also effective, precipitation being accomplished again by means of hydrochloric acid. Extractions made with distilled water in which the fiber material was heated at 75°C. for 15 minutes and allowed to stand at room temperature for 24 hours also gave abundant alcoholic precipitates, the alcohol having been acidified with 0.1 per cent hydrochloric acid. Since these procedures are representative of the standard methods of extraction of pectic substances from plant tissues, the precipitates are presumably composed to a large extent of pectic material.

The Effect of Reduced Oxygen Supply on the Germination of Cocklebur Seed. *N. C. Thornton, Boyce Thompson Institute for Plant Research, Inc., Yonkers, N. Y.*—Non-dormant embryos of the upper and lower seeds of the cocklebur, when held from 4 to 18 weeks at 28° to 31°C. under conditions limiting the oxygen supply below that normally necessary for germination by storage in hydrogen, nitrogen, or carbon dioxide or a mixture of these gases, will show various depths of delay in germination when removed to air. As long as the seed coats remain intact under favorable moisture conditions, these dormant upper and lower seeds may be kept moist in Petri dishes without germinating for as long as a year at the stated temperature. Upon removal of the seed coat, there results a delay in germination of as much as one month. Upon planting in soil after germination, a dwarfed, curly-leaved, slow-growing plant is produced; but after about 40 days the plant begins the normal rate of growth and type of development. The induced dormancy may readily be overcome by a period of low temperature storage (3 months at 5°C.).

Fluorescence in M-Ray Research. *Dmitry N. Borodin, Biological Laboratory, Cold Spring Harbor, L. I., N. Y.*—In several methods of detecting M-rays, yeast cultures are used. In a method developed by the author, yeast cells on "yeast plates" are applied in a manner similar to that in which a photographer uses silver grain on photographic plates. Two methods of sensitization of "yeast plates" to M-rays or 1800–2800 Å.U. were used. Such a wave length produces a molecular excitement of fluorescence and makes M-rays almost visible. The wave length of 1800–2800 Å.U. is converted into a longer wave length which does affect the sensitized "yeast plate." One physical and one chemical source as "artificial" M-rays were used as well as two biological sources of that radiation. The first was a carbon arc lamp with a set of filters to eliminate all visible and all other parts of the spectrum, except 1800–2800 Å.U. The second was a chemical reaction of pyrogallol oxidation. In both cases the intensity of radiation was diminished by dis-

persion and fractioning. From these two sources of "artificial" M-rays "yeast-plates" sensitized by various known fluorescent chemicals, including many glycosides, were irradiated. Only β -glycosides with l-glucose (levorotating) and not split by yeast enzymes were found of value. The process of investigating two new glycosides was discovered, including a bright fluorescent helenin. An alkaloid from *Datura* seeds producing green fluorescence was abolished owing to its lethal effect in a useful concentration. One more unidentified chemical listed as XY is now under investigation. It does not produce visible fluorescence but invisible strongly sensitized "yeast plates."

Growth of *Euglena Gracilis* in Light. *S. H. Hutner, Cornell University, Ithaca, N. Y.*—Experiments are in progress to find a synthetic medium permitting growth of *Euglena gracilis* in the dark. The experiments, carried out in light, are based on the assumption that a medium which in light allowed as good growth as peptone in light, would be as effective as peptone in the dark. Jahn's discovery that *Euglena gracilis* in light uses glucose is confirmed. The growth-promoting quality of peptone does not reside in its mineral content, as evidenced by lack of stimulation when peptone ash is added to various mixtures of organic nutrients. *Chlorella* sp., on the other hand, when supplied with iron in available form, grows better in glucose than in peptone. The conditions for utilization of acetate and sulfur are under investigation.

The Effects of High Temperatures on Seeds. *Lauretta E. Fox, University of Illinois, Urbana, Ill.*—This investigation was undertaken to determine the nature of the changes occurring in the dormant protoplasm of seeds during heating. Reid's yellow dent corn of the 1932 crop and Burpee's brittle wax bean of the 1933 crop were used. The seeds were heated in Abderhalden's drying apparatus. Twenty-eight grains of corn or seeds of beans, weighing ten grams, were used per sample for these determinations. Preliminary experiments showed that seeds are very sensitive to the amount of heat received. The time, temperature, and conditions of heating were carefully regulated. Vitality was measured as the percentage germination, the growth of the seedlings, and the colloidal index. The colloidal index was determined by measuring the amount of colloidal material leached from the seeds into distilled water. The colloidal index was read by a Leitz nephelometer. Crocker thought that death was produced in heated seeds by a denaturing of the proteins of the protoplasm. He found certain fluctuations which he did not explain. These data show that there are definite fluctuations in the vitality of seeds treated so as to be dehydrated. These fluctuations in vitality are probably associated with the denaturing of the protoplasm. Denaturing or dehydration appears to take place in several steps.

Automatic Registration of the Desiccation of Leaves. *S. Prát, Charles University, Praha.*—S. Škramovsky constructed an apparatus—stathmograph—for automatic photoregistration of changes of weight during constant or

changed temperature (Ztschr. Physik. Chem. B, 25: 1-26, 1934). This apparatus was used for determination of loss of weight of leaves. The graph of evaporation of pure water is a straight line; but the loss of weight during desiccation of leaves is registered in asymptotic curves. Different plants have characteristic curves. Living and dead tissues (before the experiment) lose water differently (constant temperature in the interval 40°-60°C.): (1) A dead leaf loses water more rapidly than a living one (*Aesculus*). (2) Dead and living tissue desiccate about equally (*Aucuba*). (3) Leaves killed before the experiment lose water much more slowly than living ones (*Pelargonium*, *Tradescantia*, *Phoenix*, *Crassula*). When increasing temperature is applied, a break in the curves is registered between 40-50°C.

Non-Symbiotic Germination of Orchid Seed. *Lewis Knudson, Cornell University, Ithaca, N. Y.*—On the germination of orchid seed two views have prevailed. One is that penetration of the orchid embryo by the appropriate orchid fungus is essential for germination. The other view, which I proposed, is that germination of orchid seed is dependent on an external source of organic food. Various sugars have been found effective in inducing germination. The latter method of germination is termed the non-symbiotic. Within the past few years incidental observations have been made on several *Cattleya* crosses in which germination occurred without sugar being available in the culture medium, although under pure culture conditions. Instead of approximately 100 per cent germination, however, the maximum germination noted has been about 20 per cent. Furthermore, the time required for germination—that is, the appearance of the first leaf—is much greater than when sugar is provided. The fact that some germination has been noted without sugar is significant in that it suggests that the germination of seed of orchid genera may not be as unusual as hitherto believed. The evidence thus far obtained, however, is only suggestive, and such germination without sugar may be confined to the embryos of those genera which become green within ten days after the seed is placed in the culture tubes.

Giant Plastids in Ferns Produced from X-Rayed Spores. *Lewis Knudson, Cornell University, Ithaca, N. Y.*—In the course of investigations on the effects of x-rays on spore germination and gametophyte development of the fern *Polypodium aureum*, certain abnormal chloroplast behavior was noted. In one type, in prothallia from irradiated spores large amoeboid-like plastids were observed, especially in the peripheral cells of the prothallium. In the older cells of the prothallium, in the region of the rhizoids, the plastids were more uniformly circular in cross section, the average diameter being approximately 9.6 microns; but many of these plastids were of unusual size, some being 22 microns in diameter. In the peripheral cells the amoeboid-shaped plastids were of various grotesque forms. At times only one single plastid with an area of the cell would be noted. Sometimes two or three plastids

would be observed, more or less rectangular in shape and having the area of the cell. In other cells adjacent one large plastid 30 microns in its greatest dimensions would be noted, without smaller plastids 5 to 8 microns in diameter in the same plane. In the very early stages of germination such abnormal plastids were evident. Even in the second or third cell the plastids were large—9 microns in diameter as opposed to the normal-sized plastid of a diameter of from 3 to 4 microns—and with further development of the protonema, plastids were noted with diameters of 15 to 20 microns. This condition is apparently fixed, for pieces of prothallium transferred to other tubes regenerated to yield prothallia with like plastids. These transfers have been continued for over two years. Photographic and other evidence reveal that the large giant amoeboid or plasmodial-like plastids are formed by the union of the smaller plastids. In turn the giant amoeboid or plasmodial-type plastid by a budding process gives rise again to the spherical plastids which have diameters two to three times the diameter of the usual plastids in this fern. Thus far only a few sporophytes have been produced from these prothallia, but examination of the few leaves thus far investigated reveals that the sporophyte has the same large plastids common to the mature cells of the gametophyte.

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